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ALLAN WINTER ROWE

Dr. Rowe, the 15th President of the Association for the Study of Internal Secretions and Director of Research of the Evans Memorial, Boston, died of aplastic anemia on December 6, 1934. For the past five years he had been Chairman of the Council.

Dr. Rowe was a native of Gloucester, Massachusetts, where he was born July 31, 1879, the son of Arthur and Lucy (Winter) Rowe. Entering the Massachusetts Institute of Technology he was graduated with the class of 1901, receiving his B.S. in chemistry. Thereafter he was deeply interested in all that pertained to Technology and had done more for the athletic life of the institution than almost any other single individual. He was secretary of his class, for many years he had been a member of the advisory council on athletics, and for some time was its secretary-treasurer.

Following his graduation from Technology, Dr. Rowe carried on research work at Wesleyan University for two years, and received his M.S. there in 1904. For two years he was in Germany, having won a Swett Fellowship, and at the University of Gottingen he won his Ph.D. degree in 1906. Returning to this country, Dr. Rowe was appointed professor of chemistry at Boston University and about the same time was affiliated with the graduate school of Harvard and for several years he carried on research work with the late Professor T. W. Richards. He also for a time was chemist at the Massachusetts Homeopathic Hospital.

Dr. Rowe was an honorary fellow of the American Medical Association and the Massachusetts Medical Society; a fellow of the American Academy of Arts and Sciences and of the American Association for the Advancement of Science; a member of the American, French and English Chemical Societies, the American Physical Society, the American Physiological Society and the Society for Experimental Biology and Medicine.

He was the first honorary secretary of the International Society for Anaesthesia Research, and was a member of Theta Xi and Phi Beta Kappa fraternities. He was a trustee of the Boston Psychopathic Hospital.

Dr. Rowe lived a very full life. His genius for friendship, his kindliness and sincerity were known and appreciated by many more friends than it falls to the lot of most people to have. Gallantly he lived and gallant was his going.

IN COMING ISSUES

The following manuscripts are in hand for publication in early issues:

Physical associations in adults with behavior problems. A. W. Rowe and Miriam Van Waters, Boston.

Thyroid and senescence. Structural transformations of the thyroid in old age and their functional interpretation. G. C. Dogliotti and G. Nizzi Nuti, Florence, Italy.

The anterior pituitary hormone content of the hypothalmus of dogs. Giacomo Pighini, Reggio Emilia, Italy.

The effect of spaying on body growth and the organ weights of the albino rat. Clay B. Freudenberger and Oscar A. Billeter, University of Utah, Salt Lake City.

The concentration of gonad-stimulating hormone in blood serum and of oestrin in the urine throughout pregnancy in the mare. H. H. Cole and F. J. Saunders, University of California, Davis.

Concerning the anterior pituitary-gonadal inter-relations. Warren O. Nelson, University of Missouri, Columbia.

Experiment to produce lactation in castrate women. August A. Werner, St. Louis.

Changes in the anterior hypophysis of the male albino rat after castration and experimental cryptorchidism. E. T. Ellison and J. M. Wolfe, Nashville, Tenn.

Endometrial studies. G. S. McClellan, Doris Phelps and John C. Burch, Nashville, Tenn.

Results of preoperative administration of an extract of pregnancy urine: A study of the ovaries and of the endometria in hyperplasia of the endometrium following such administrations. E. C. Hamblin, Durham, N. C.

A case of pituitary infantilism treated with commercial anterior pituitary preparations. George B. Dorff, Brooklyn, N. Y.

The relation of blood volume to certain glands of internal secretion. Effect of thyroid and gonad ablation. Mae Friedlander, Norman Lasky and Samuel Siebert, New York.

Changes in the endometrium after female sex endocrine therapy: A consideration of the effect of antuitrin-s in human subjects. John Rock, Brookline, Mass.

Experiments with theelin and galactin on growth and function of the mammary glands of the monkey. Edgar Allen, W. U. Gardner, and A. W. Biddle, New Haven.

Acquired resistance to the thyroid-stimulating and pseudoluteinizing hormone of cattle anterior pituitary. Paul Max, Mary M. Schmeckebier and Leo Loeb, St. Louis.

The effects of pituitrin, pitressin and pitocin upon the copper reducing substances in the serum and urine of dogs. A. R. McIntyre, R. F. Sievers and H. F. Elias, Omaha.

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CONTEMPLATING THE HORMONES*

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COLD SPRING HARBOR, N. Y.

Present pursuit of the hormones is at such a pace that we may well be persuaded that endocrinologists are just now in a bigger hurry than any other group of investigators in the world. In this we are right: never before were so many choice secrets of chemical regulation spread just beyond our finger tips. However, from all this important activity of a recent period I am here regretfully obliged to refer merely to some fragments which to me seem of rather special significance to both biology and medicine.

THE CHEMICAL TRANSMISSION OF NERVE IMPULSE EFFECTS

First among things of such significance let us place the now fairly clear demonstration that the nerve impulses of the autonomic nervous system obtain their responses from smooth muscle, cardiac muscle and many glands only through chemical mediators produced at the junction of the fiber with the muscle or gland cell. Further points in this development include the fact that one type of substance—very like adrenine—is produced at the endings of most sympathetic fibers, while at the endings of parasympathetic fibers and of certain sympathetic fibers a quite different substance—acetylcholine—is produced; also included in this development is the high probability that both of these "chemical mediators" must be considered true hormones, and indeed perhaps more ancient in animal history than any hormone that we now know. Though the studies which lead to these and other important related conclusions are the work of many men during two or three decades it is work of the past year or two which has brought most elements of definiteness and clarity into the picture. Fortunately both Cannon (1) and Dale (2), whose laboratories are contributing so much in this field, have very recently given authoritative summaries of these facts in lectures which will doubtless soon be in print.

My purpose here is to bring some of these prime conclusions momentarily into this meeting of the Association and to consider the high place these results assume in the present picture of hormonal regulation.

*Dinner address, read before the Eighteenth Meeting of the Association for the Study of Internal Secretions, Cleveland, June 11, 1934.

The substance given off at the endings of most sympathetic fibers was called "sympathetic substance" by earlier workers and Cannon introduced the more convenient word, sympathin. Chemical and spectrographic tests by Bacq have recently shown that sympathin is a catechol derivative with an aminated side-chain, and therefore it is either adrenine or a very closely related substance. Cannon and Rosenblueth, however, obtained results which they interpret as showing that sympathin and adrenine are not exactly the same, though they produce identical effects under nearly all of the conditions investigated. Moreover, Cannon finds reason to assume that the sympathin formed at the ends of nerve fibers produce excitation or inhibition by union with a hypothetical substance existing in the innervated cell; and that in cells that are excited this substance is of Type E, while in cells that are inhibited it is of Type I. Thus, sympathetic impulses ultimately produce both sympathin E and sympathin I.

The studies of Cannon and associates have made it quite certain that sympathin can be carried by the blood-stream and produce throughout the body its characteristic effects—those of sympathin E or of sympathin I—on organs capable of giving the appropriate response to this adrenine-like substance. Sympathin, again like adrenine, does not long remain in the circulation, but its stability is similar to that of adrenine.

Let us next for a moment turn our attention to the substance produced at the ends of parasympathetic nerve fibers. It has now been made practically certain that this substance is acetylcholine—a substance which was shown by Reid Hunt many years ago to be about 1,000 times more active than choline. The substance in question is definitely proved to be an ester of choline, and acetylcholine is the only such ester that has yet been found in the body. The great instability of acetylcholine in blood or tissues proved to be practically an unsurmountable obstacle to a proper study of this substance until it was learned that the special enzyme, esterase, which hydrolyses it can be satisfactorily inhibited by small amounts of eserine (physostigmine). With the protection afforded by eserine recent work, particularly in Dale's laboratory, has shown that acetylcholine occurs normally in the spleen and placenta—where its physiological function is not yet clear—and that it is produced at the endings of stimulated parasympathetic fibers and of some sympathetic fibers as well. Since some fibers arising from sympathetic ganglia thus produce acetylcholine at their distal terminals Dale proposes a classification of autonomic nerves based on their functions rather than their anatomic relationships; thus, on a functional basis we have "cholinergic" and "adrenergic" nerves. Throughout the autonomic nervous system, then, a chemical mediation of nerve impulses is now found; and acetylcholine and sympathin are those mediators.

Curiously enough Feldburg, Minz and Tsudzimura (3) find that when the splanchnic nerves supplying the suprarenal medulla are stimulated, in order to increase the output of adrenine, acetylcholine appears in the suprarenal vein, thus indicating that it is acetylcholine which transmits to the medullary cells their stimulus to excrete adrenine into the blood.

Remembering that these medullary cells are by derivation the equivalent of sympathetic ganglion cells, it is both interesting and important to note that evidence (Feldburg and Gaddum) is already at hand indicating that the transmission of a nerve impulse across a synapse in a ganglion—the superior cervical ganglion was used—is effected by the release of acetylcholine. On this point Dale states that "it is clear that when preganglionic impulses arrive in the ganglion, acetylcholine is there released in such an amount that it not only may, but must stimulate the ganglion cells to their only known form of activity, in the output of impulses in the postganglionic fibers, corresponding to those which arrive in the preganglionic fibers."

These and still other results provide exciting indications that chemical mediation may soon be found to play a clarifying part in the operations of the central nervous system itself. Again Dale and Feldburg, and others, have already found some evidence that acetylcholine is also produced in the transmission of a motor nerve impulse to a voluntary nerve fiber. Such a wide-ranging series of results enormously expands our past conception of the intimacy of the association between chemical (hormonal) and nervous regulation.

A word concerning the adaptiveness and evolutional significance of these two substances is warranted. According to Cannon, "it is noteworthy that sympathin, the chemical representative of sympathetic impulses, is much more stable than acetylcholine, the representative of the parasympathetic impulses. Whereas the former can readily be shown not only to be present in circulating blood and to cooperate with adrenine, and to have wide-spread effects in the body, the demonstration of acetylcholine in the circulation is difficult and requires specially devised conditions to prolong its normally brief existence. This striking difference between the two mediating substances is quite in harmony with evidence as to the difference of neural organization of the sympathetic and the parasympathetic divisions of the autonomic system. The sympathetic neurones are organized for diffuse discharge of their impulses through the organism, to produce related adjustments of bodily processes—a provision favored by coöperation of sympathin and adrenine simultaneously set free in the blood stream and acting, therefore, diffusely. The parasympathetic neurones, on the other hand, are arranged for specific effects on separate organs—constriction of the pupil, for example, and slowing of the heart. These effects are quite unrelated. If acetylcholine were a persistent agent in the blood, it might bring about temporally related changes in biologically unrelated phenomena. Fortunately that result is avoided by the extreme instability of the mediator of parasympathetic impulses."

Dale has tentatively surveyed the various functions of the entire nervous system with a view to estimating the relative extent to which each type of chemical mediator has been utilized by the nervous mechanism. On this point he states that "we get an impression of the cholinergic mechanism as having the more general application in the functions of the nervous

system, and probably an earlier origin in evolution, and of the adrenergic mechanism as a more specialized and probably a more recent development."

In the course of the development of our knowledge of sympathin and acetylcholine the term hormone has only infrequently been applied to them —particularly in the case of acetylcholine; more cautiously and more often they have been called merely "chemical transmitters of nervous effects"—a thing which they indisputably are. Writing nearly two years ago, Babkin and associates (4) expressed the view that further studies on the cholinergic principle would have to decide whether or not this substance, then less well identified than now, is or is not a true hormone. At present, it seems to me that absence of histological or neurological data to show that bodies comparable to myoneural junctions really exist at the ends of many autonomic fibers is the main or only objection to granting acetylcholine a place among the true hormones. And, the absence of such specific tissue may not be a reality, but reflect merely the failure of neurological technique to demonstrate it. Since the adrenergic principle is either adrenine itself, or is perhaps only debatably distinct from it, its hormonal status is hardly in doubt.

To all those therefore who earnestly press for an understanding of the regulatory mechanisms of the body—and to those who find very special interest in the place of hormonal regulation in the organism—these superb results, giving the hormones a share in much of nervous action, now set new outposts on a frontier whose further conquest is the concern of us all.

A POINT OF DIFFERENCE BETWEEN NEURAL AND HORMONAL REGULATION

During the past years of swift strides in endocrinology it was inevitable that some tentative consideration should be given to the question of specific reasons for two types of regulatory systems—nervous and endocrine—found in all higher animals. Contemplating this question for a moment is perhaps permissible here and now, since the studies which have just been reviewed have a bearing on the problem. We all know that a very simple and distinct form of "chemical" regulation antedated in the animal series both the nervous and hormonal types of regulation. But which came first, nerve or true endocrine? And why any kind of highly evolved regulation other than nerve regulation?

Several years ago I (5) hazarded on these points some expressions which in the main are perhaps supported by later work, but which in part require reconsideration. The new facts concerning adrenine and acetylcholine carry with them a strong probability that they are older in race history than any other hormones now known to us. If this is so, perhaps it can be said that their association with nerve fibers suggests only that hormones and nerve *fibers* are equally or indistinguishably ancient. The literal acceptance of that indication, however, involves a suggestion that the still earlier fiber*less* stages of the evolution of the nerve cell—those logically necessary stages involving concentration or accentuation of the properties of irritability and conduction in the simplest ancestral nerve

cells—would still precede the origin of hormones. Again, to me it appears unlikely that the "neurofibrils" now found in some single-celled animals would at all require the aid of a true hormone in carrying out their simple and seemingly obvious functions. Just here there is much speculation and little evidence, but an estimate of which came first, nerve or endocrine, is certainly as desirable as it is dubious.

There remains the question—and to investigators of hormone action it is a question even of practical importance—why is not all regulation *nerve* regulation, since nerve cells send their fibers to every millimeter of living tissue. There is, of course, the well-recognized reason that certain slow and enduring responses are better and more economically accomplished by hormones. But for myself the opinion has become conviction that the nervous system—even with the aid of a hormone at endings of its fibers—is by its own *nature* quite incapable of regulating certain types of activity. What, then, are such types of activity?

I wish to cite irregular and regional breaks of the steady state (homeostasis) as such a type. I think it is now evident that marked irregular and regional breaks in the steady state, most notably those which involve temporary or repetitive developmental (growth, differentiation) change, are—despite the usual need for homeostasis and the numerous mechanisms invoked to maintain it—the work of hormones. Also, that wherever we encounter such phenomena there is presumptive evidence of their hormonal control. It is indeed true that some hormones usually, and very effectively, help to maintain homeostasis, not the opposite; adrenine, thyroxine, the pituitary posterior lobe pressor principle, cortin, and probably others could be cited as *usually* helping to maintain the steady state. Yet, even some of these usually smooth-running hormones may, so to speak, "have their moments"—and break their custom to the ultimate and very great advantage of the organism.

Nowhere so well as in the reproductive system can we illustrate and contrast the nerve and hormonal types of activity. The nerves at all stages of life doubtless hold their grip on the regulation of blood supply to gonad, uterus and mammary; they share largely in the control of the composition of that blood; probably their impulses prevent atrophy of some essential structural elements, though this does not apply to smooth muscle; and probably nerve impulses also control in some cases the time and amount of hormone released for their most disruptive tasks. But the actual speedy and spectacular changes in gonad, uterus and mammary, are all the direct and special work of hormones. Follicle-stimulating pituitary hormone, estrin, progestin, and prolactin—all exerting their essential action in the precise order named—effect in mammals, in a way well known to this group, the metamorphosis of a near-infantile or inactive state to a fully functional state. These developmental changes in specifically limited areas are real and highly advantageous escapes from homeostasis, and they are at the same time unique presentations of an aspect of the growth process.

In our egg-laying vertebrate ancestors other and often even more profound transformations of hormonal origin occur in conjunction with mere ovulation. In pigeons at this period the blood calcium is more than doubled, the blood sugar is increased during eight or nine days, the uterus undergoes a ten-fold increase in size, and very marked histological changes occur in the thyroids and suprarenals. Nearly all of these changes have also been observed in fishes or frogs and in fowls. In these cases we deal with the more or less brusk transformations typically effected by hormones. The nervous system, aided indeed by some hormones, usually powerfully drives for homeostasis; by contrast, and equally serving the preservation of the organism, many hormones temporarily and cyclically produce amazing changes within localized well-guarded areas.

Finally, and from a different standpoint, the neuron is a transmitter, the endocrine organ a giver. When an endocrine cell prepares molecules which it has unique powers to put together and then continuously gives these to a stream that takes them to a distant universe of otherwise talented brothers, the procedure appears to transcend charity and become pure benevolence. We, of course, know that disease, as well as tonic life, may go on the wings of those freely-given molecules; but the endocrine characteristically "gives," and it is good to find in these bits of flesh with which we work the hint of a quality which we should so much like to find in ourselves.

PUTTING THE THYMUS TO WORK

There are new reasons for admitting the thymus into the family circle of busy hormones. Better still, on this point the new data of Rowntree, Clark and Hansen (6) are of such nature that they will not fail to gain the attention of clinicians and biologists generally. But let us first note one or two items in the development of the story of the endocrine function of the thymus.

Twenty years ago Gudernatsch (7) probably correctly observed that at a particular phase of the tadpole's development the growth processes are specifically accelerated by feeding the animals on thymus tissue. Ten years ago, after having obtained evidence that the nerves do not control those particular secretions, I could show (8) that the formation of egg-albumen and egg-shell which certain birds place around their egg yolks depends upon a product of the thymus. Certain data connected with our tests made it very improbable that this product was of the nature of a nutrient. The evidence of its hormonal nature was presented, and for it the name *thymovidin* was suggested. No extracts were made and none were necessary since, in the peculiar test animals used by us, dried ox thymus was fully effective when given by mouth. The facts observed very greatly impressed me—though as yet there is little indication that they have impressed others —with the importance of the thymus for building the egg-envelopes which all of our sub-mammalian vertebrate ancestors must build in order to develop or to exist at all.

Four years ago Asher (9, and later papers) could show that a substance obtained from the thymus had growth-promoting power in rats, and for this substance he introduced the name, *thymocresin.* Whether thymovidin, thymocresin, and the effective substance in Hansen's extract as now used by Rowntree and Clark, are the same substance or are different is quite unknown.

Very recent results obtained by Rowntree and Clark are, however, as astonishing as is all else connected with the investigation and the neglect of this obtrusive and peculiar organ. Still more striking than their results recently published in abstract are additional data received in personal communication a week before this was written. In the fourth and fifth generations of injected rats the teeth erupted in less than 24 hours after birth, and this was 9 to 10 days in controls; the eyes opened on the second to third day (fifth generation), instead of at 14-17 days; the animals were covered with fur by the second or third day, instead of at 14-17 days as in controls. The young of the fifth generation are weaned when 2 or 3 days old, and "at 6 days they can swim like muskrats and seem as competent in water as in their cages."

Rowntree records the intriguing observation that the accruing acceleration of growth and development is slow in its advent—that is, this is not striking before the third or a later generation of treatment—but that this accrual of growth disappears much more rapidly when treatment is discontinued. "If, for example, three generations are treated and one generation left without treatment their offspring revert to almost normal rates of growth and development." It is noted, too, that the treated adults are similar in body size to the controls; it is very early, preadolescent growth, not ultimate size, that is affected.

"The time of the onset of adolescence and maturity has also been advanced in a striking manner. Thus in the fifth generation adolescence has been attained in the male from the fourth to the eighteenth day as compared to the normal, 40 to 90 days; and in the female from the 20th to the 25th day instead of the 70th to the 90th day. In addition to precocious adolescence, fertility has also been increased."

To this notable statement by these workers—a statement which we all hope will soon be suitably consolidated by them—I can here add only an idle thought. Mammals develop their eggs within their bodies; they must *not* develop emphatic egg-envelopes. To their advantage mammals can lose, or somehow avoid, the thing which I have regarded as the primary thymus function of building egg-envelopes—a function which was species-preserving in their ancestors. But incident to the reduction of this function there may have been consequences—even including unessential temporary handicaps. Perhaps mammals and humans can propagate their kind without the primary thymus hormone—though I have often wondered whether a certain type of human sterility will not some day be found to be associated with a generalized deficiency of lymphoid (and thymus) tissue.

We now ask, is the substance which controls the building of egg-envelopes (this probably is produced by lymphoid tissue generally, not by thymus alone) the same substance that has power to add to speed of adolescence and to speed of preadolescent growth? If so, we may suspect that unrestricted hereditary losses or reductions of thymovidin in mammals resulted from the circumstance that such losses are here not vital, and probably not even disadvantageous to individuals which must long develop in a womb and then get out of it. This point is capable of at least a partial experimental test. If the acceleration phenomena observed by Rowntree and Clark in the rat can be duplicated in man and mammals generally, but cannot be obtained in birds, that suggestion of an hereditarily reduced thymic function in mammals would be supported. In this connection I cite an earlier personal observation (10) that in birds—I have since seen this in several wild species—thymic and related tissue exists in very extraordinary volume during very early life; that this very early period of life is a period of phenomenally rapid growth; and finally, that thymic tissue there begins to involute as the growth curve begins to flatten.

From these worthy data and my own very idle remarks about them we, the vainest of the primates, could begin to suspect that our conventional but low-ebb thymus may have been doing some bruskly restraining things to our own racial and very personal history—such, for example, as a sharp rationing of our early growth, and a resolving of our early years into what is really a prolonged and stunted epoch. Perhaps our prolonged infancy and childhood are not "deficiency phenomena" such as medicine will often be called upon to mitigate. But the results already obtained on the rat signal a triumph—they seem to say that to a few individuals of one mammalian species the prying investigator has just now given back something which, millions of years ago, the rigorous demands of the mammalian type of reproduction took away.

THE ATTACK ON THE ANTERIOR PITUITARY

From this point onward in this discussion I ask your permission to speak of situations and problems connected with present attempts to understand the ways and work of the anterior pituitary gland—a subject on which a generous amount of new light has come during the past year. It is a splendid record. Moreover, a situation without parallel in the investigation of any other endocrine organ has recently developed in connection with relatively early attempts to isolate and determine the number of distinct anterior pituitary hormones. This work is, for most of us and for the immediate present, as helpful and progressive as it is also baffling and confusing. The laboratories of Evans and Collip particularly have not only got growth, gonad-stimulating, prolactin and thyreotropic principles; they, or others, get parathyreotropic, adrenotropic, fat-metabolism and luteinizing principles. Thus in the work of various laboratories not fewer than eight distinct principles are proposed. As already indicated, it is still too early, even for a participant in anterior pituitary study, to venture a

statement concerning this galaxy of hormones; and Collip (11) has expressed well a point of reserve on this matter. Personally, I would care to say only that I suspect that further study is much more likely to reduce than to increase the above list of pituitary principles; and, that part of a seeming multiplicity is largely connected with the circumstance that, in the case of the anterior pituitary, biochemistry is finally being forced to deal with a mixture of perhaps four hormones of complex protein nature.

Workers represented here would disagree as to which are the outstanding developments in pituitary work during the past year. Somewhere very high among the most notable things will have to be placed the emergence of the pituitary effect on the thyroid into proofs that this effect is produced by a true and separate anterior pituitary hormone. For several years Aron (12) and others have urged that the thyroid-stimulating principle of the pituitary is separate from the others, but the earlier data fell far short of proving that this is so. The really decisive evidence has slowly accumulated. The thyroid responses have probably all been obtained with impure preparations, none of which was free from follicle-stimulating hormone and most of which contained at least another principle. On the basis of personal tests of many of these preparations, we doubt that any thyreotropic preparation has ever been made which did not contain fair amounts of the follicle-stimulating principle. We have assayed the chief European and American preparations for their follicle-stimulating potency, and have found it in all. Corroborative and decisive evidence for the separateness and individuality of the thyreotropic factor has therefore had to come from various sources, most of which cannot be noted here. On this point our laboratory—and here is included the work of Drs. Bates and Moran, and of Lahr and Smith—has shown that prolactin does not mediate this thyroid response. We also find (unpublished data) that mare serum, which is known to have very high gonad-stimulating potency and negligible effects on thyroid histology, has also no effect on the basal metabolic rate of either normal or hypophysectomized pigeons.

Those who work directly with the pituitary hormones have recently been stimulated by data which Collip and associates (13) have obtained and interpreted as indicating the experimental production of "antihormones." Without venturing an opinion on this difficult subject—Dr. Collip will later present further original data at this meeting—we can say that, in the case of prolactin, we early found that its stimulating effect on the crop-gland of the pigeon could not for long be sustained at a high level. The effectively stimulated gland will soon go into decline despite continued dosage, and the reason for this unexpected refractoriness of the organ remains for us a challenging problem.

In anterior pituitary work I think our primary obligation is to find out which specific processes and tissues are stimulated by each of the now known pituitary hormones, and a brief survey of this point must provide a conclusion for the present discussion. We shall travel slowly by injecting hormonal *mixtures*, noting some direct or indirect response in some part of

the organism, and then naming a new pituitary hormone for the newly observed response. We shall move steadily on firm footing by learning precisely which functions and tissues are directly affected by each particular hormone. This necessarily follows from the circumstance that hormone action is highly specific action. It is worth while to consider how much of really specific action is now known for some of the anterior lobe hormones.

First, the follicle-stimulating hormone. Until very recently it was not positively known that the true follicle-stimulating hormone acts upon a specific part or tissue of the gonad. Such specific action of the hormone was theoretically to be expected; but from the early days of this work "luteinization" accompanied follicular growth when extracts of either pituitary tissue or urine were administered, and confusion and uncertainty as to which and how many tissues and causative agents were involved were things which kept us from knowing the specific point of action of the true follicle-stimulating hormone. Recently and quite decisively, Smith, Engle and Tyndale (14) have shown that the pure follicle-stimulating substance can be recovered from castrate and menopausal urine, and that the action of this substance is definitely limited to the germinal tissue of the gonad. Fevold and Hisaw (15) and Wallen-Lawrence (16) have reported a separation of both a true follicle-stimulating and an exclusively luteinizing fraction from pituitary tissue. The nature and other properties of this luteinizing fraction, and other contaminating principles present in the two fractions, are indeed not yet known.

Second, the thyreotropic hormone. The known point of primary action is the secretory tissue of the thyroid. It, of course, remains to learn conclusively whether other tissues or functions are directly stimulated by this same hormone, but Collip, Anderson and Thomson (17) seem to have proved that this hormone does not also stimulate the suprarenals.

Third, prolactin. Certainly fair progress has been made in defining the processes governed by this hormone in higher vertebrate animals. Reluctant human lactation has been adequately stimulated by prolactin preparations heated to boiling for one hour, and the fully prepared mammary glands are its specific point of known action in mammals—from opossum to human. In pigeons it specifically acts upon the crop-glands (and broodiness?), and evidence was obtained that there it also has specific power to diminish the germinal tissue of the gonad. This same action was found likewise in the gonad of the fowl, but we should note our inability to diminish testis size in either old or adult rats with prolactin. This failure is probably connected with the circumstance that the testes of adult and old rats cannot be stimulated to excess size by follicle-stimulating hormone—according to Cole and others (18), Evans and colleagues (19), and according to our own unpublished studies. Again, in fowls our laboratory finds (20) that broodiness is a specific state induced by prolactin. Since some reptiles brood their young we thus have indications that prolactin has a history reaching at least into the middle of the series of vertebrate animals.

Fourth, and finally, let us consider the growth hormone and related responses. In some respects it is almost unique and anomalous that there should exist a hormone which has *no* specific point of action, but which—like a vitamine—contributes to the growth of *all* tissues; and which, in such animals as fishes and reptiles, presumably remains active to the end of very long life, while in other animals its period of action is limited to early life. Of course, it is probable that thyroxin affects the rate of oxidation in practically all tissues, and that cortin likewise affects one or more cellular (or are these extracellular?) processes perhaps throughout the body, etc.; but in these cases it is doubtful whether anything so hugely inclusive as normal or supernormal *body growth* is ever contributed by a single hormone.

In an earlier period of study various investigators, notably Evans and Cushing, valiantly strove to associate the growth hormone with the stimulation of one or more of the endocrine glands—the thyroid and the suprarenal in particular. But stimulation of the thyroid by the pituitary is now seen to be the work of what we have been speaking of as the thyreotropic hormone. Should this have been called the growth hormone? Certainly we know that cretins grow under oral administration of the thyroid hormone. And to this should be added the case of the dwarf mice studied by Smith and MacDowell (21). These mice have deficient eosinophile-pituitaries, thyroids and adrenals. Such mice were later shown by MacDowell and Laanes (22) to be capable of good, not best, growth when given desiccated thyroid alone. Further unpublished studies, in which our laboratory has collaborated with MacDowell and Laanes, have sought an analysis of the growth effects of the individual pituitary hormones in this extremely favorable animal. Since the preparations used on the dwarfs were assayed for organ responses on immature pigeons, and for basal metabolic rate on other pigeons, those results are of real interest.

We note the following: The follicle-stimulating hormone of mare serum, and that in pregnant urine, neither stimulates growth in the dwarf nor raises the B.M.R. in pigeons. Good thyreotropic preparations, though fairly rich in follicle-stimulating hormone, bring good but not full growth in the dwarf, and those preparations raise the B.M.R. in pigeons. Prolactin has a poor but definite effect on growth in the dwarf, and definitely increases the B.M.R. in pigeons. When prolactin and thyreotropic (containing also follicle-stimulating from pituitary) were given in combination they gave more growth than when each was given alone; and when these preparations had been heated to boiling for one hour their growth-promoting effects were very nearly as great as before heating. Phyone—which we know contains follicle-stimulating, thyreotropic, prolactin, and doubtless other material not present in highly purified preparations—gives the best growth of all in the dwarfs; and it gives the highest B.M.R. in the pigeons. The heating of this phyone to 80° C. for 15 minutes markedly reduces its growth-promoting potency.

Knowing the necessity for repair of both suprarenals and thyroids in these dwarfs, and observing the additional growth which thyreotropic-free

prolactin brings to a thyreotropic preparation, and finding best growth in a mixed "growth" preparation (phyone), are things suggesting that normal growth in these dwarfs is largely the sum of the contributions of more than one hormone—perhaps of one which repairs its thyroid, another which repairs its suprarenals, and yet another which supports some growth-limiting process or organ; probably a residue of growth-promoting power lost through heating the phyone is still left unaccounted for. Which hormone, then, is here stimulating the adrenal? Is this prolactin, or is it an adrenotropic hormone of the pituitary which we have been unable to exclude completely from our prolactin preparations? Perhaps it is not prolactin, but at the moment we are interested in the consequences of its being either prolactin or a separate adrenotropic principle. If the growth hormone repairs neither the suprarenal nor the thyroid, and no specific point of action be found for it, what is its status and how may we surely recognize it in our preparations?

It appears that some heat-labile substance, not necessary for *good* growth but for the *best* growth, was present in the "growth hormone" preparations used here by us, and as used earlier and elsewhere by many others. But it has now become fairly clear—the point is attested by Collip (11) and by Evans (23)—that adrenotropic and thyreotropic effects are not mediated by the growth hormone; and we have just observed in a pituitary-deficient animal that more than one pituitary hormone has growth-promoting power. Does the growth-promotion induced by a distinct growth hormone begin only where the growth resulting from the full function of thyroid and adrenals—and perhaps of yet another organ or organs—leaves off? Further proofs on the nature, point of action and individuality of the "growth hormone" are now of capital importance in current study of the anterior pituitary.

SUMMARY

Recent work on sympathin (Cannon) and acetylcholine (Dale) is considered in its special relation and significance to endocrinology.

Neural and hormonal mechanisms of regulation are compared. It is noted that temporary regional breaks in homeostasis, particularly those involving temporary or cyclical developmental (growth and differentiation) processes, cannot be directly mediated by nervous action and are under hormonal control.

Aspects of recent thymus investigation (Rowntree) are discussed in their relation to earlier thymus study. It is suggested that Rowntree's results on the rat may indicate that the mammalian type of reproduction has permitted, even required, hereditary losses from the very high functional level of the ancestral thymus.

Recent developments in anterior pituitary study are examined critically, and unpublished data on several topics are presented. Only very recent study adequately proves the existence of a distinct thyreotropic hormone. The reality of the alleged great multiplicity of anterior lobe hor-

mones is questioned. In pituitary-deficient dwarf mice more than one pituitary hormone has been shown to have growth-promoting power. Further proofs on the nature, specific point of action and individuality of the "growth hormone" are regarded as of capital importance in present study of the anterior pituitary.

REFERENCES

1. Cannon, W. B.: The story of the development of our ideas of chemical mediation of nerve impulses. Am. J. M. Sc. (In press.)
2. Dale, H. H.: Chemical transmission of the effects of nerve impulses. Brit. M. J. (In press.)
3. Feldburg, W., B. Minz and H. Tsudzimura: J. Physiol. **81**: 286. 1934.
4. Babkin, B. P., A. Alley and G. W. Stavracky: Humoral transmission of chorda tympani effect. Trans. Roy. Soc. Can. **26**: 89. 1932.
5. Riddle, O.: On endocrines and organisms. Am. Naturalist, **61**: 481. 1927.
6. Rowntree, L. G., J. H. Clark and A. M. Hanson: The biological effects of thymus extract. Proc. Am. Physiol. Soc. **109**: 90. 1934.
7. Gudernatsch, F.: A further contribution to the knowledge of organs with an internal secretion. Am. J. Anat. **15**: 431. 1914.
8. Riddle, O.: A hitherto unknown function of the thymus. Am. J. Physiol. **68**: 557. 1924.
9. Asher, L.: Der Einfluss der Thymus auf das Wachstum und die Herstellung eines wirksamen Thymusstoffes Thymocrescin. Endokrinologie, **7**: 21. 1930.
10. Riddle, O. and P. Frey: The growth and age involution of the thymus in male and female pigeons. Am. J. Physiol. **71**: 413. 1925.
11. Collip, J. B.: Chemistry and physiology of anterior pituitary hormones. Trans. Congr. Am. Physicians and Surg., 15th Session. 1933.
12. Aron, M.: L'Hormone prehypophysaire excito-secretrice de la thyroide. Rev. franc. d'endocrinol. **6**: 472. 1930.
13. Collip, J. B. and E. M. Anderson: The production of serum inhibitory to the thyreotropic hormone. The Lancet, **1**: 76. 1934.
14. Smith, P. E., E. T. Engle and H. H. Tyndale: Gametokinetic action of extracts of follicle-stimulating urine. Proc. Soc. Exper. Biol. & Med. **31**: 745. 1934.
15. Fevold, H. L., F. L. Hisaw, A. Hellbaum and R. Hertz: Sex hormones of the anterior lobe of the hypophysis. Am. J. Physiol. **104**: 710. 1933.
16. Wallen-Lawrence, Z.: Proof of the existence of a follicle-stimulating and a luteinizing hormone in the anterior lobe of the pituitary body. Proc. Soc. Biol. Chem. **105**: xciii. 1934.
17. Collip, J. B., E. M. Anderson and D. L. Thomson: The adrenotropic hormone of the anterior lobe. The Lancet, **2**: 347. 1933.
18. Cole, H. H., H. R. Guilbert and H. Goss: Further considerations of the properties of the gonad-stimulating principle of mare serum. Am. J. Physiol. **102**: 227. 1932.
19. Evans, H. M., K. Meyer, M. E. Simpson and others: The growth and gonad-stimulating hormones of the anterior hypophysis. Mem. Univ. of Cal. **2**: 446 pp. 1933.
20. Riddle, O., R. W. Bates and E. L. Lahr: Prolactin induces broodiness in fowls. Am. J. Physiol. (In press.)
21. Smith, P. E. and E. C. MacDowell: An hereditary anterior pituitary deficiency in the mouse. (Abstract.) Anat. Rec. **46**: 249. 1930.
22. MacDowell, E. C. and T. Laanes: Carnegie Institution of Washington, Yearbook No. 31, p. 47. 1932.
23. Evans, H. M.: Present position of our knowledge of anterior pituitary function. J. A. M. A. **101**: 425. 1933.

COMPOUNDS THAT AFFECT THE BASAL METABOLISM IN MAN†

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THYROXINE AND RELATED COMPOUNDS, INCLUDING DINITROPHENOL

The past thirty years have witnessed the isolation and synthesis in pure form of several compounds (presumably catalysts) which have been demonstrated to affect in different manners the rate at which various chemical changes occur in the cells of the body. The mechanisms by which these alterations are brought about remain obscure, although some progress has been made in the realm of oxidation-reduction phenomena. In Table 1 we have compared the calorigenic effects of some of these compounds. Of the substances compared, thyroxine is the most powerful calorigenic agent. Thus, the intravenous administration of a single dose of 10 mg. to a patient whose basal metabolism is from 40 to 45 per cent below normal will raise the rate 32 points on the average in from three to ten days; and the effect on metabolism lasts from 70 to 90 days (1) (2). There are reasons for believing that the thyroid of a normal man produces thyroxine or its equivalent at the rate of about 0.3 mg. per day (3) (4), and that there are about 10-14 mg. in the body outside of the thyroid gland (4) (5). If slight alterations are made in the thyroxine molecule, the calorigenic effects of the products are much smaller than that of thyroxine. Observations with diiodotyrosine, thyronine, diiodothyronine and N-acetyl thyroxine illustrate the importance of the diphenyl ether group, the amino group and all four iodine atoms (6) (7). Diiodotyrosine, because of the nature of its formula, is thought to be the precursor of thyroxine (8). It may have a slight calorigenic effect, which, on the basis of the observations reported here, is about 1/9900 as great per millimol as thyroxine given intravenously. If a phenol group is added to the molecule with the production of diiodothyronine, the calorigenic effect is increased about 25 times, diiodothyronine having about 1/400 as much effect as thyroxine per millimol, in terms of extra calories, but about 1/36 as much effect in terms of the amount of increase in the basal metabolism. This discrepancy may be explained by the fact that after a single dose of diiodothyronine the effect on metabolism lasts for only seven to fifteen days compared with 70 to 90

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days for thyroxine. In other words, by removing two atoms of iodine from the thyroxine molecule, not only is the effectiveness of the compound in raising metabolism greatly diminished, but the nature of the metabolic response is altered. If the two remaining atoms of iodine are removed, the resulting compound, thyronine or desiodothyroxine, is virtually inert, 228 mg. having had no effect in one patient and 400 mg. no effect in another. The iodine in the molecule is, therefore, of the greatest importance in the production of calorigenic activity. If one of the hydrogen atoms in the amino group of thyroxine is replaced by an acetyl group to form N-acetyl thyroxine, the calorigenic activity is also reduced. This compound, when given intravenously has, per millimol, about 1/13 as much effect as thyroxine given intravenously; and, when given subcutaneously, about ¼ as much. Kendall (9) and Swingle, Helff and Zwemer (10)

TABLE 1

COMPARISONS OF CALORIGENIC ACTIVITY OF VARIOUS COMPOUNDS

Compound	Molecular Weight	Dose Mgm.	Basal Metabolic Rate Before Administration	Excess Calories Produced	Excess Calories Per Mgm.	Excess Calories Per Millimol
Adrenalin (Boothby and Sandiford [13])	183	0.5	....	25	50.0	9,150
Thyroxine (synthetic)	777	10.0	−39	17,135	1,715.0	1,331,390
Diiodotyrosine	433	8600.0	−35	3,165	0.4	135
Thyronine*	273	400.0	−37	0	.0	0
Diiodothyronine	525	150.0	−40	940	6.0	3,310
N-acetyl Thyroxine—						
Intravenously	819	31.5	−41	4,030	130.0	104,830
Subcutaneously	819	31.5	−34	11,480	365.0	298,445
Alpha Dinitrophenol 1:2:4	184	400.0	−25	745	2.0	340

*It would, of course, be necessary to administer much larger doses of thyronine over a long period to be sure that it is entirely devoid of calorigenic activity.

All administrations, not otherwise noted, were intravenous, except adrenaline which was given subcutaneously and dinitrophenol, which was given by mouth.

All compounds were given in single doses except diiodotyrosine, which was given in 16 injections over a period of 18 days, and thyronine, which was given in 2 injections on successive days.

have reported this compound to be without any effect in man, but to produce metamorphosis in tadpoles; while Gaddum (11) found that in large doses it did produce an increase in metabolism in the rat. Thus, when there is carried out what may be termed a chemical dissection of the thyroxine molecule, it appears that every part is essential for maximum activity.

In view of the importance of the iodine in the thyroxine molecule, it is of interest that dinitrophenol, which contains only one benzene ring and no iodine in the molecule, possesses well-marked calorigenic activity, although producing only about 1/3900 of the effect of thyroxine per millimol. However, the nature of the metabolic response is entirely different from that of thyroxine. After a single dose the effect on metabolism becomes maximum within five hours and lasts only from four to five days. The curves for dinitrophenol and diiodothyronine are somewhat similar, but a longer time is required for the metabolism to rise to a maximum after

diiodothyronine and slightly longer for it to return to its original level. The beneficial and other clinical effects that follow the administration of thyroxine are virtually absent following the administration of dinitrophenol. Thus a patient may continue to be myxedematous in spite of the fact that the basal metabolism has been increased from minus 40 per cent to normal. This amount of change in metabolism produces little change in pulse rate and the increased nitrogen excretion which follows the administration of thyroxine is said to be absent following the administration of dinitrophenol (12). These experiments with dinitrophenol suggest that different types of alteration in metabolism may be present in spite of the same total rate of oxygen consumption.

The effect of adrenaline [figures from Boothby and Sandiford (13)] is about 1/145 of that of thyroxine per millimol. It is the quickest acting of the substances listed, its effect becoming maximum within from 15 to 30 minutes and disappearing within 60 minutes. Its effect is also different clinically from that of thyroxine. Thus we have four different chemical substances which produce entirely different types of metabolic responses, adrenaline, thyroxine, diiodothyronine and dinitrophenol. In general, it would appear that the more rapidly the effect becomes maximum the more rapidly it disappears.

In recent years glutathione has attracted considerable attention because of its supposed rôle in oxidation-reduction phenomena in cells. It would be desirable to know whether it plays any rôle in the action of thyroxine. Alper and Thompson (14) have accordingly given it in small doses shortly preceding and following the administration of a single dose of thyroxine without any clear-cut effect. We have also given small doses to two patients with exophthalmic goiter without any clear-cut change in metabolism. As progress is made in our knowledge of tissue oxidations we shall undoubtedly know more about the mechanisms by means of which thyroxine produces its effect. Because of the slowness with which its effect becomes maximum and the slowness with which it disappears, it is desirable to know whether thyroxine takes part in the chemical reactions which follow its administration, or whether it sets in motion chemical changes which continue some time after it has disappeared from the reaction.

DERIVATIVES OF DESICCATED THYROID

One problem of the greatest importance is the rôle thyroxine plays in the functioning of the normal thyroid gland. It has been rather generally taught that thyroxine is the only compound in the gland which possesses calorigenic activity. Some investigators have found that the activity of thyroid is proportional to its thyroxine content and have suggested that for pharmaceutical purposes desiccated thyroid should be standardized on this basis (15) (16). We have recently collected some data which have an important bearing on this matter (7). We have found that after heating for four hours on a water bath with a small amount of normal sodium hydroxide, the calorigenic activity of desiccated thyroid is reduced by more

than two-thirds. Thyroxine itself is unaffected by the same treatment. There is no reduction in activity if the mixtures are not heated. Regardless of the theoretical explanation of these findings, they show that the procedure which is common to all methods for determining the amount of thyroxine in the thyroid, namely, hydrolysis with alkali, destroys most of the gland's activity. Kendall (17) has sometimes been unable to isolate any thyroxine from thyroid glands which possessed well-marked physiological activity. The low yield of thyroxine from desiccated thyroid observed repeatedly by Kendall (17) and by Harington (8) may well be related to the effect of heating with alkali.

Another problem which has been attracting attention for a considerable period is the relation between the calorigenic activity and the total organic iodine content of the gland. Chemical evidence favors the view that a large part of the iodine in the thyroid possesses little or no calorigenic activity (8) (18). This comparatively inert fraction is thought to be in the form of diiodotyrosine. We have found that, after digestion with pepsin§ most of the calorigenic activity of desiccated thyroid is possessed by the acid-insoluble fraction, containing about half of the total iodine. Per mg. of iodine, this fraction appears to possess greater calorigenic activity than the desiccated thyroid from which it is prepared. In one patient 3.1 mg. of iodine in the form of the acid-insoluble precipitate administered by mouth produced nearly as much change in metabolism as 6.5 mg. intravenously in the form of thyroxine. A small amount of activity is possessed by the acid-soluble fraction after peptic digestion. It appears to produce greater intoxication than the acid-insoluble fraction and the curves denoting the change in metabolism after its administration are characterized by a more rapid return to the level before treatment. It thus appears that there may be two compounds in the thyroid with different calorigenic activities.

PITUITARY AND THYROID

Of particular interest is the demonstration that the anterior lobe of the pituitary contains a substance which produces an increase in metabolism in animals when administered parenterally. This increase has always been temporary and has been reported not to occur in the absence of the thyroid. Hyperplasia of the thyroid and exophthalmos have been reported in association with the high level of metabolism in ducks (19) and guinea pigs (20) and the high metabolism is said to be reduced by iodine (21). Attempts to reproduce in man the results observed in animals have been disappointing, but we have had the good fortune in the past six months to obtain two extracts of the anterior lobe of the pituitary which possess calorigenic activity. One is the growth hormone of Squibb's and the other the product of the Wilson Laboratories, known as "Phyone."‡ These extracts have produced an increase in metabolism in 24 of 39 cases

§We wish to thank Dr. Klein of the Wilson Laboratories for enzymes and desiccated hog thyroid supplied for these experiments.

‡We are indebted to Dr. Morrell and Dr. Klein, respectively, for supplying these extracts.

in which they have been tried. In every instance the response has been temporary, the metabolism finally returning to its original level in spite of continued treatment. Of particular interest is improvement in Simmond's disease, increase in metabolism in patients with mild myxedema who have non-toxic goiters, and an increase in the severity of exophthalmic goiter followed by a reduction in severity after omission of the extract. The patient with Simmond's disease was of interest because his clinical condition resembled in some respects that seen in nephrosis. On admission to the hospital there was emaciation, weakness and pitting edema, which was generalized but most marked in the legs; a concentration of protein in serum of 4.8 per cent, of calcium 8.4 mg. per 100 cc. and of sugar, 66 mg. per 100 cc.; a normal blood pressure; and only a slight trace of albumin in the urine from time to time. With rest in bed and two large doses of thyroid the edema subsided and some improvement occurred. The improvement became still more marked when his metabolism rose from minus 30 per cent to plus 5 per cent during the administration of the pituitary extract, but the improvement tended to disappear as his metabolism dropped, in spite of continued treatment. When his metabolism was at the high level, the concentration of protein in serum was 6.2 per cent and of calcium 9.7 mg. per 100 cc., but it is impossible to tell whether this change was caused by the pituitary or the thyroid.

In a patient with a large colloid goiter and a basal metabolism of minus 25 per cent that we have thought to be caused by an underfunction of the thyroid, the rate rose to plus 12 per cent during the administration of the pituitary extract, in association with some decrease in the size of the thyroid. When the extract was omitted the metabolism fell to its level before treatment at about the same rate as would be expected after omission of thyroid. When desiccated thyroid was administered to the patient, the goiter decreased in size markedly. In other words, if it be true that the pituitary affects metabolism only through the thyroid, then in this case, a thyroid gland which was producing an inadequate amount of its hormone could be made to produce an adequate supply by an outside stimulus.

In a patient with a non-toxic adenomatous goiter, one of the extracts produced an increase in basal metabolism from minus 7 to plus 24 per cent, in association with the development of symptoms suggesting mild thyrotoxicosis, namely, tachycardia, slight nervousness and sweating. These symptoms disappeared when the metabolism returned to normal.

In a patient with exophthalmic goiter there was a well-marked increase in most signs and symptoms of the disease during the increase in metabolism, associated with the administration of the extract, and a decrease in the intensity of the disease clinically when the metabolism dropped after omission of the extract.

In the four patients with well-marked myxedema and presumably no functioning thyroid tissue to whom we have administered the extracts, there has been no increase in metabolism. However, in two cases the doses

were too small to conclude that the pituitary does not produce its calorigenic effect in the absence of thyroid function. Should this prove to be the case, then it becomes apparent that the functional activity of any thyroid tissue that is present may be greatly altered by some stimulus from the pituitary.

SUMMARY

Thyroxine possesses more calorigenic activity than any other compound so far isolated in pure form. Other compounds affect the basal metabolism per millimol in the following order: N-acetyl thyroxine, adrenaline, diiodothyronine, dinitrophenol and diiodotyrosine. Thyronine appears to be inert.

Observations on diiodotyrosine, thyronine, diiodothyronine and N-acetyl thyroxine show that slight changes in the thyroxine molecule greatly reduce its calorigenic activity and in particular that the amino and diphenyl ether groups and all four iodine atoms are important. Removal of two iodine atoms to form diiodothyronine not only greatly reduces its effectiveness in raising metabolism, but markedly shortens the duration of the calorigenic response.

After a single dose of dinitrophenol the metabolism becomes maximum within five hours, compared with two days for diiodothyronine and from three to ten days for thyroxine; and returns to its pre-treatment level in about three to five days compared with seven to fifteen days for diiodothyronine and 70 to 90 days for thyroxine (basal metabolism minus 40 per cent).

The effect of adrenaline becomes maximum within 15 to 30 minutes and disappears within 60 minutes. It would appear that the more rapidly the calorigenic effect of a compound becomes maximum, the more quickly it disappears.

All methods used to determine the thyroxine content of desiccated thyroid destroy most of its calorigenic activity.

By digestion of desiccated thyroid with pepsin, an iodine fraction has been obtained (acid-insoluble) which appears to possess greater activity per mg. of iodine than the whole gland from which it was prepared. By the same procedure another fraction has been obtained (acid-soluble), which produces an effect on metabolism which, per mg. of iodine, is of much smaller magnitude and of much shorter duration.

Following the subcutaneous administration of two different extracts of the anterior lobe of the pituitary, a clear-cut increase in basal metabolism has been observed in 24 of 39 cases of various types.

REFERENCES

1. Thompson, W. O., P. K. Thompson, L. F. N. Dickie and S. G. Taylor: West. J. Surg. **41**: 431. 1933.
2. Thompson, W. O., P. K. Thompson, A. G. Brailey and A. C. Cohen: J. Clin. Investigation, **7**: 437. 1929.
3. Boothby, W. M., I. Sandiford, K. Sandiford and J. Slosse: Tr. A. Am. Physican, **40**: 195. 1925.

4. Thompson, W. O., L. L. McLellan, P. K. Thompson and L. F. N. Dickie: J. Clin. Investigation, **12**: 235. 1933.
5. Plummer, H. S.: J. A. M. A. **77**: 243. 1921.
6. Thompson, W. O., S. B. Nadler, S. G. Taylor and P. K. Thompson: Proc. Soc. Exper. Biol. & Med. **31**: 1000. 1934.
7. Thompson, W. O., P. K. Thompson, S. G. Taylor, S. B. Nadler and L. F. N. Dickie: J. A. M. A. In press.
8. Harington, C. R. and S. S. Randall: Biochem. J. **25**: 1032. 1931.
9. Kendall, E. C.: Chemistry of the Thyroid Secretion. The Harvey Lectures, Series **15**: 41. 1919-20.
10. Swingle, W. W., O. M. Helff and R. L. Zwemer: Am. J. Physiol. **70**: 208. 1924.
11. Gaddum, J. H.: J. Physiol. **68**: 383. 1930.
12. Cutting, W. C. and M. L. Tainter: J. A. M. A. **101**: 2099. 1933.
13. Boothby W. M. and I. Sandiford: Am. J. Physiol. **66**: 93. 1923.
14. Alper, J. and W. O. Thompson: Unpublished data.
15. Gutman, A. B., E. M. Benedict, B. Baxter and W. W. Palmer: J. Biol. Chem. **97**: 303. 1932.
16. Harington, C. R.: The Thyroid Gland, its Chemistry and Physiology. London, Oxford University Press, 1933.
17. Kendall, E. C.: Thyroxine. The Chemical Catalog Co., New York. 1929.
18. Harington, C. R. and S. S. Randall: Biochem. J. **23**: 373. 1929.
19. Schockaert, J. A. and G. L. Foster: J. Biol. Chem. **95**: 89. 1932.
20. Friedgood, H. B.: Bull. Johns Hopkins Hosp. **54**: 48. 1934.
21. Friedgood, H. B.: Personal communication.

TWO CASES OF CHILDHOOD MYXEDEMA, REPORTED FOR THE PURPOSE OF EMPHASIZING THE IMPORTANCE OF BONE-AGE STUDIES*

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Childhood myxedema is a clinical syndrome, due to absence of, or deficiency of, the internal secretion of the thyroid gland, having its onset at some time prior to adolescence. As in the adult form, the incidence is greater in females than in males. In a series of one hundred cases collected from the North American Literature, Gordon (1) found that in seventy per cent the onset could be traced to between six months and two years of age.

Endocrinopathies arising in infancy, childhood and adolescence demand careful study of the osseous system. In 1896 Hertoghe (2) pointed out the retardation of carpal development which accompanied childhood hypothyroidism. Later Dieterle (3) compiled a table showing the normal age for appearance of the various bones of the hand; this constituted the first step in the appreciation of "Bone-age" studies. In 1924 Engelbach and McMahon (4) stressed still further the great significance and value of roentgenographic studies to determine bone-age by compiling a chart for the entire osseous system, giving the time of appearance of the various nuclei and the age of closure of the various epiphyses. A more elaborate presentation of this diagnostic aid can be found in Engelbach's recent volumes on Endocrine Medicine (5). Shelton (6) has more recently contributed his share to this diagnostic maneuver.

By utilizing these charts one can readily ascertain which regions must be x-rayed for diagnostic purposes and further, by comparison with the Table of Normals, determine the degree of osseous retardation present in a given case. Periodic x-rays of a case of hypothyroidism while under treatment, provides another "measuring stick" for observing progress of the patient.

While the function of the thyroid gland plays a very significant rôle in osseous development one should not be unmindful of the various other factors involved in the development of the skeletal system.

This question of "Bone-Age" studies from a differential diagnostic standpoint was presented by one of us (7) in 1929 and is herewith briefly summarized. The osseous system is influenced by:

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1. Endocrine conditions which produce premature ossification.
2. Endocrine conditions which retard ossification.
3. Non-endocrine conditions or diseases which may alter ossification.

Endocrinopathies which stimulate premature ossification include tumors of the pineal, suprarenal cortex, ovary and testicle. All of these produce rapid skeletal growth or apparent gigantism, but likewise result in premature closure of the epiphyses, culminating therefore in normal or even subnormal stature; a kind of transitory gigantism. Inasmuch as these several tumors may evoke an identical osseous response, recognition of the gland at fault must depend upon other localizing evidence. This need not be considered here.

Endocrinopathies producing retarded ossification are:

1. Childhood myxedema in which there is delay both in appearance of bone nuclei and in epiphyseal union. Bourneville reported a remarkable instance in which all epiphyses were found open, at autopsy, in a patient aged 36 years.

2. In hypophyseal infantilism, in all likelihood due to inadequate secretion of the growth-stimulating and maturity-provoking hormones from the adenohypophysis, ossification is usually retarded, sometimes markedly. Ordinarily, however, this delay in appearance of bone nuclei is not as striking as in primary thyroid failure. Other clinical features, such as absence of adiposity, gracile skeleton and infantile genitalia, help to distinguish this group. We have been interested in a lad age 17 who was an excellent example of pituitary infantilism; his bone-age was 13.

3. Preadolescent eunuchism or eunuchoidism, in which the sex glands are either absent or deficient, is characterized by prenounced lateness of epiphyseal closure. As a consequence such boys or girls are apt to continue growing at an age when growth has ordinarily ceased. Usually, therefore, they become quite tall, though rarely gigantic; disproportionately long extremities as compared to the trunk is typical for this endocrine disturbance. Thus one of us (H. L.) had the opportunity of studying a girl aged 24 years who was five feet ten inches tall. She had never menstruated and the secondary sex characteristics were completely absent; her bone age was only 12. In a few instances the epiphyses have remained open throughout "adult" life. We found the epiphyses ununited in a man aged 44 who in all other respects also was an outstanding example of eunuchoidism originating in childhood.

Consideration must be given to a group of developmental abnormalities for which an endocrine causation has not yet been proven.

1. True Infantilism, which Falta describes as a standing still at the infantile stage of development, with deficient growth, delay in appearance of bone nuclei and epiphyseal closure. The patient retains childish skeletal dimensions, childish genitalia and childish psyche. The endocrine system participates in, but does not originate this condition.

2. Primordial Dwarfism is a congenital condition in which the infant is dwarfed at birth. Development proceeds normally as to appearance of bone nuclei and closure of epiphyses. The individual matures sexually and intellectually but remains dwarfed.

3. Paltauf's Dwarfism. These children are normal at birth; suddenly cease growing in youth and later in life may suddenly start growing again. Genitalia and secondary sex characteristics are retarded; bone nuclei are retarded and epiphyses may remain open into late adult life.

4. In Ricketic Dwarfism there may be slight variation either in the direction of osseous retardation or acceleration. Intelligence and sex development are normal. One can always find evidence of previous rickets in these cases.

5. The Achondroplastic Dwarf develops normally both intellectually and sexually. The condition is characterized by disproportionately short extremities. The ossification of the epiphyses may vary, some closing prematurely, others late.

6. In Mongolian Idiocy there is usually some stunting of growth, slight delay in appearance of bone nuclei, but no delay in epiphyseal closure. The condition is readily recognized by the almond-eyed slant, retarded mentality, and an incurvation of the shortened little finger.

Since the thyroid hormone plays such an important rôle in the development of the osseous system, a deficiency prior to adolescence will give roentgenological evidence of its presence to a degree depending upon two factors: first, the age of the patient at the onset of the malady; and secondly, the degree of thyroid deficiency. While it is true that the table of standards for a given age is subject to some variations, it is as valuable in diagnosis as a basal metabolic rate or any other laboratory procedure, all of which must be tempered by clinical judgment. We now have at our disposal a simple means of diagnosing hypothyroidism in infancy and certainly this is the ideal time to start treatment for at this time we find the minimal discrepancy between chronological age and mental age.

As was recently stated by one of us (8): Inasmuch as respiration chambers are not generally available for basal metabolism estimations in infants and the ordinary machines cannot be utilized for children under five or six years of age; and, since the standards for older children are not altogether accurate; and lastly, since most infants and many children suffering from myxedema or moderately severe hypothyroidism are mentally deficient and, therefore, unable to coöperate in the performance of a basal test—for all these reasons it is fortunate that another technical procedure is available, and is of extraordinary usefulness in recognizing preadolescent thyroid deficiency, namely, the determination of osseous age by roentgenograms. Finally it may be added, that the taking of roentgenograms is far more trustworthy, and much more comfortable for the child, than basal metabolism tests.

The first case to be presented unfolds the signs and symptoms of childhood myxedema so completely that the case report itself suffices admirably.

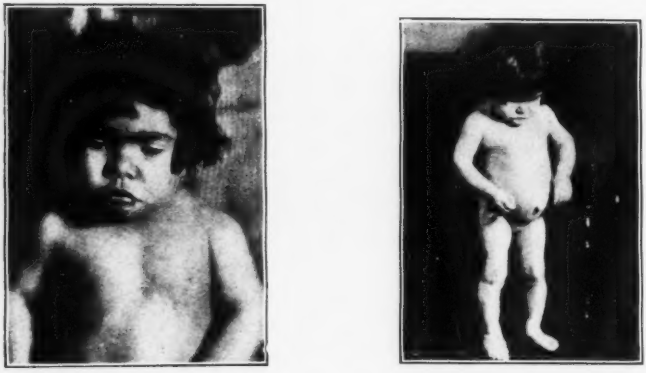
Case 1. Childhood myxedema in a girl aged 12, showing extreme osseous retardation.

This patient (R. G.) is the fourth child in a family of six, born to normal parents. All the other children are within the limits of normality.

The father is 5 feet 3 inches in height and weighs 145 pounds, and the mother is 4 feet 11 inches and weighs 104 pounds. Both parents are of Italian descent and lived in the South until they came to California thirteen years ago.

In spite of her age of twelve years, the patient was brought into the office, carried in the arms of her mother. She was not able to stand alone for more than a few moments and was able to take only a few tottering steps. Her expression was dull and stupid and her complexion was sallow or pasty.

She is said to have weighed ten pounds at birth but there was no difficulty at the time of delivery. She cut her first tooth at the age of three; first attempted to stand at the age of five and was still unsteady when she tried to stand or walk. She was constipated, having a bowel movement only once in four to six days.



A B

Figure 1. (a) (Case 1). Childhood myxedema in girl aged 12 years. Bone-age: 6 months to 1 year. Note stupid expression, abundant coarse straight hair, heavy eyebrows, puffiness around eyes, saddle-nose, thick lips and protruding tongue. (b) Note marked stunting of growth. Height: 30 inches. Weight: 29 lbs. Note absence of breast development, short stubby fingers, bracelet fat padding, protuberant abdomen, umbilical hernia and extreme degree of flattening of arches of feet.

Her life up to this time had been practically a vegetative existence for she was quite content to pass the whole day either in her crib or sitting on the floor—showing but little interest in her surroundings. She could drink from a glass but usually had to be fed.

We were fortunate in obtaining some clinical records which show that at 2 years 7 months she was 26½ inches tall and weighed 18 pounds. At 5 years 9 months she was 28 inches tall and weighed 21 pounds. At this time an attempt was made to determine the I. Q. and she was rated as having a mental age of one year.

When first observed by one of us (C. K. C.), she stood 30 inches in height and weighed 29 pounds; her temperature was 97.2, rectally, and the pulse rate was 72. Her hair was coarse and black and straight, extending well down her already low forehead. The eyes could scarcely be seen through the narrow lid slits, which were almost completely closed by the puffiness of the lids and surrounding tissues. The eye-brows were heavy and were well knitted in midline. The broad saddle-nose contributed its share to the characteristic picture [Figure 1 (a)]. The lips were thick and pouting and were perpetually parted by her protruding boggy tongue which apparently could not in its entirety find accom-

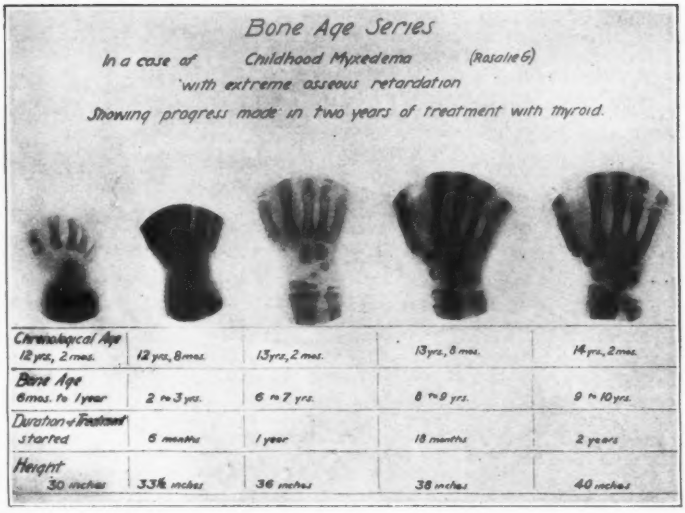


Figure 2. (Case 1). Note progress made in osseous development during 2 years on thyroid treatment.

modation in her buccal cavity. The hard palate was broad and flat and there were but eight teeth in her mouth; four permanent molars and four deciduous cuspids; although the permanent incisors were in the process of erupting.

The thorax was well proportioned and the nipples were absolutely flat upon the chest wall; there was not the slightest indication of mammary development. The abdomen was of the pot-belly type and a large ventral and umbilical hernia were clearly evident [Figure 1 (b)]. The external and internal genitalia were infantile.

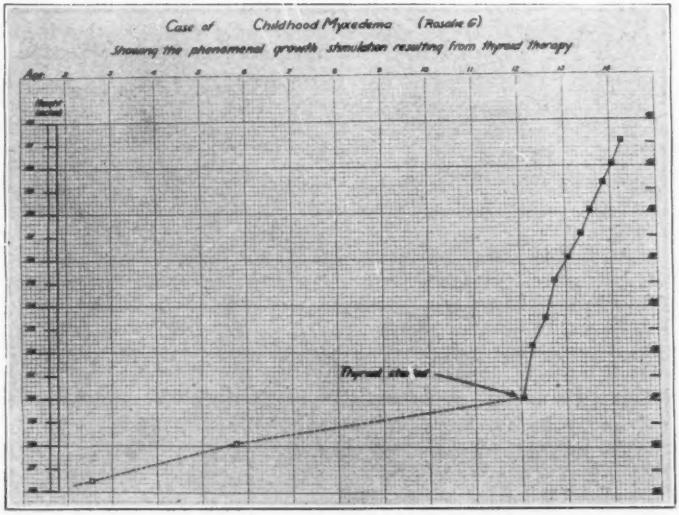

Case of Childhood Myxedema (Rasalie G)
Showing the phenomenal growth stimulation resulting from thyroid therapy
Age:
Thyroid started

Figure 3. (Case 1). Note growth-stimulating effect of thyroid treatment. Growth during 27 months on thyroid: 11 inches. Growth during previous 10 years: 4 inches.

The hands were small, with broad, tubular, stubby fingers and the wrists were broad and showed classical bracelet fat padding. The feet were flat to an extreme degree and this was exaggerated still further by the infantile fat pads which were still present beneath the arches. The toes displayed the same broad, stubby appearance that was observed in the fingers.

At this time another attempt was made to determine the I. Q. and she was again given a rating of one year (11 years mental retardation).

Since a basal metabolic test was impossible in this case an x-ray determination of bone-age was made to obtain further information regarding the degree of thyroid deficiency. This showed the presence of but two of the carpal bones, giving her a bone-age of but 6 months to one year; an astounding retardation of over 11 years (Figure 2). X-rays of the thymus and sella turcica showed both to be normal. Examination of the urine gave normal findings and the Wassermann reaction was negative. The hemoglobin was 50 per cent and the red blood cells numbered 2,100,000, revealing a severe degree of anemia with a high color index. The white blood cells amounted to 8,000 with 42 per cent neutrophiles, 57 per cent small lymphocytes and 1 per cent eosinophiles.

Having established the diagnosis of severe childhood myxedema, she was placed on desiccated thyroid, one grain daily. The mother was instructed to give the child no other medication, not even laxatives.

Almost like magic, this child began immediately to take on a new lease on life and proceeded, as it were, to come out of hibernation.

At the end of three months she had grown 2¼ inches and lost 3 pounds; the puffiness around her eyes was rapidly disappearing and the eyes were bright; her expression was one of alertness. Her lips became less prominent and the boggy tongue was beginning to show some muscular tone. Her bowels were now moving once a day. An x-ray study for bone-age at this time showed an advancement to between 1 and 2 years. She now was actively interested in play about the house and yard and for the first time walked into the office with a fairly steady gait.

An attempt was made at this time to increase the dosage of desiccated thyroid to one and a half grains daily but on account of the mother's persistent reports of the child's nervousness and restlessness at night, the dosage was again reduced to one grain daily.

At the end of six months the mother reported that the child was happy and at play all day long. Her feet had grown so that she now required size 6 shoes, whereas she had worn size 4 for several years prior to the present treatment. The edema of the face had melted away and she had grown a total of 3½ inches. Her hair had lost its coarse, straight, dry appearance and her skin was now warm and moist to the touch. At this time her bone-age had advanced still further to between 2 and 3 years (Figure 2).

Nine months had elapsed since the treatment was instituted when the patient was two hours late in keeping her appointment at the office. Her older sister apologized for the tardiness and explained it by stating that the patient had insisted on going to a "Movie" before coming to the office; an amazing change from her previous languor. At this time she stood 35 inches high and had a bone-age of 3 to 4 years.

In one year of treatment her bone-age had advanced from 6 months to between 6 to 7 years (Figure 2). She grew 6 inches in one year whereas she had grown less than 4 inches in the previous ten years (Figure 4). During this same year her brother, who is 19 months younger, grew three inches and her brother who is three years her senior grew 2½ inches.

The patient has now been under treatment a little over two years and has grown 11 inches. Her bone-age has advanced to 9 years, which constitutes an osseous increment of eight years in a time interval of two years (Figures 2, 3 and 4).

Summary of Case 1

A girl of 12 years, with a bone-age of 6 months and an I. Q. of one year, infantile in stature and behavior, who had grown but 3½ inches in the previous ten years, grew 11 inches in two years on thyroid therapy; her bone-age advanced from 6 months to 9 years and her behavior was strikingly transformed.

Comment. This girl had been seen in a clinic when she was six years old; at that time she was correctly recognized as a case of childhood myxe-

dema and placed on thyroid therapy. She was kept under observation for three months and then failed to return. She received no further treatment until seen by one of us two years ago. There is every reason to hope for a continuation of growth up to such time as her epiphyses close. As far as her mentality is concerned, six very valuable years have been lost. It is very problematic how much mental improvement can be hoped for after her brain has been dormant for twelve years.

When a definite diagnosis of childhood myxedema is made, a condition for which we have a simple, inexpensive, and easily administered specific form of therapy, we have a duty to sufficiently enlighten the parents so that no further time is lost in attempting to restore the child to a normal

Case of Childhood Myxedema (Rosalia G.)

showing the transformation wrought in this child during 27 months of thyroid therapy.

Before starting treatment. After 27 months of treatment.

	Age	Bone Age	Height		Age	Bone Age	Height	Growth
Patient (in center)	12yr, 2mos.	6mos-1yr.	30 ins.		14yr, 5mos.	9-10yrs.	41 ins.	11 inches
Brother (left)	10yr, 7mos.		52 ins.		12yr, 10mos.		56¾ ins.	4¾ ins.
Brother (right)	15yr, 2mos.		61 ins.		17yr, 5mos.		64½ ins.	3½ ins.

Figure 4. (Case 1). Note transformation in 27 months on thyroid substance. Growth of patient during this period: 11 inches. Growth of her brother on the left: 4¾ inches. Growth of her brother on the right: 3½ inches.

status. This is not always a simple matter, for in spite of the marvelous response shown by this patient it became necessary for one of us on many occasions to call for this child personally and bring her to the office in order that periodic observations might be made. It is true that after one year of treatment had been completed and the results were so apparent, no further difficulty was encountered in obtaining the utmost coöperation from the parents, but the critical time is the first few months of care when the progress is more apparent to the trained medical observer than it is to the parents who have long since resigned themselves to the child's fate and scarcely dare hope that this curious mass of humanity, might, under proper guidance, be molded into normal human form.

Case 2. A case of childhood myxedema discovered in a woman at the age of 34.

This patient (B. W., unmarried female, aged 34) came to the clinic complaining of stiffness of her knees. Since about six years she had noticed that when walking up or down stairs her knees were a little stiff and weak; no difficulty was noted when walking on the level, unless she had been seated for a long period of time and then attempted to walk. The condition had developed gradually; there had never been any pain, swelling nor redness of the knees or any other joints.

She was born in Western Texas and lived there until three weeks prior to coming under our observation. During all but the summer months she was definitely more sensitive to cold temperatures than were other members of her household.

She is said to have weighed 10 pounds at birth and has always been somewhat overweight. At ten months she started to walk; and started to school at

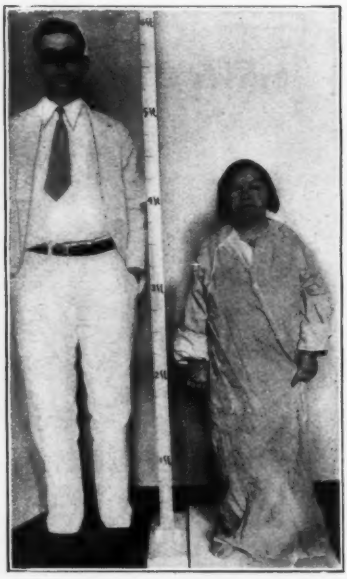


Figure 5. (Case 2). Childhood myxedema in woman aged 34 years. Note short stature, puffiness of face and hands, and moderate obesity. Height: 4 ft. 4 in. Weight: 107 lbs. Bone-age: 10 years.

the age of six, continuing through the eight grades of Common School without much difficulty, although she was never very fond of studies. Since leaving school she occupied herself keeping books, playing the piano and selling tickets in her father's show house.

There was never any noticeable difference in her height as compared with her school mates until about the age of twelve, when all the other children seemed to "grow right away from her," and she has not grown since that time. Her skin has always been dry and flaky and she perspires only in the very hottetst weather. For the past year she has had some brownish spots on her face. Her health had been unusually good, having had measles and whooping-cough in childhood and on no other occasion had it been necessary for her to seek the services of a physician. She was 16 or 17 at the time of the onset of her menstrual periods; the interval has never been regular, varying from one month to one year; the flow was moderate in amount, lasting four or five days.

Both parents were born in Mississippi and moved to Texas after their marriage. The mother was 5 feet 9 inches tall and was of slender build; she died

at 59 from heart failure. The father is living and well. He is 5 feet 10 inches tall and is of average build. The patient has two sisters and one brother, all of average height and apparently free from any stigmata of endocrine dyscrasias.

The patient was a short, obese, coöperative female of limited intellect, modest and shy to an extreme degree. Her voice was decidedly deep and of a dry, leathery coarseness and she walked with a pronounced waddle. She stood 4 feet 4 inches tall and weighed 107 pounds (Figure 5). Her pulse rate was 60, and the blood pressure 105/60.

Her moon-shaped face was puffed out with a non-pitting edema and the skin was coarse and dry and almost leathery in appearance. The hair on the scalp was abundant but straight, coarse and dry. The eyes were almost closed by the puffy red cheeks which, in contrast to the pale surrounding skin, looked rouged, giving that well-known "Apple Blossom" complexion of myxedema. The eyebrows were thinned and absent in the outer thirds. The nose had a broad, flat bridge and flaring thick alae. The lips were protruding and thick and the tongue was broad and flabby (Figure 7).

The neck was short and fat, forming a pronounced double chin. Some areas of brownish pigmentation were present on the neck and in the coarse, deep wrinkles of the face and neck. There were prominent fat pads in the supra-

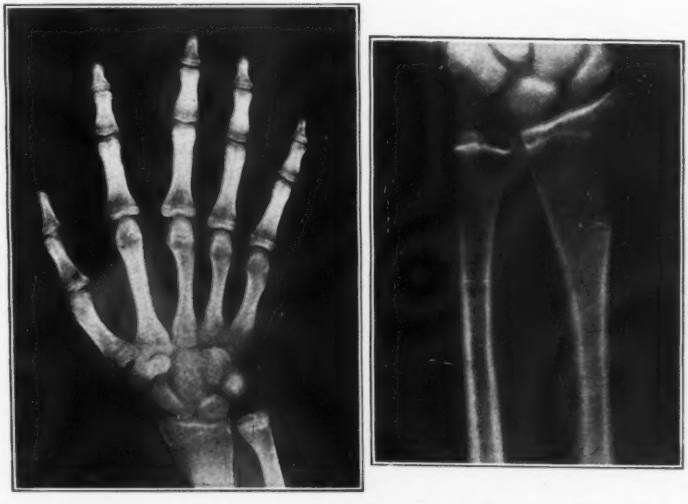


Figure 6. (Case 2). X-rays of hand and forearm for bone-age studies. Chronological age: 34 years. Bone-age: 10 years. Epiphyseal lines of metacarpals and phalanges still distinctly visible although united. Distal epiphysis of ulna still clearly separated. All epiphyses are still open, except olecranon of ulna and lower end of humerus.

clavicular and nuchal regions. The breasts were large and pendulous. The abdomen was protuberant and very thick. An umbilical hernia was present and there was a definite separation of the recti muscles, especially at the upper portion of the abdomen. Pubic and axillary hair were absent.

The hands and feet were broad and stubby and the nails were extremely brittle. There was no limitation of motion of the knee joints; all reflexes were equal and normal. The basal metabolic rate was 32 per cent minus; this was repeated a few days later and was 39.5 per cent minus. The paleness of the skin and mucous membranes was substantiated by a hemoglobin of 60 per cent and red blood cells numbering 3,560,000. Her apparent mental sluggishness was confirmed by an I. Q. determination which gave her a rating of 12 years 1 month. Bone age studies in this case required x-rays of practically all the bones of the skeletal system. The epiphyses of the metacarpals and phalanges were still distinctly visible, although united (normally unite during the 14th or 15th year). The distal epiphysis of the ulna was still clearly separated (normally unites at the 7th year). All epiphyses were still open except the olecranon of the ulna and the lower end of the humerus—the bone-age was therefore estimated as about 10 years (Figure 6). Her blood serum calcium was 13.45 mg. per 100 cc.

The glucose tolerance test gave blood sugar values of 100, 142, 149 and 111 mg. per cent. Wassermann reaction was negative and the urine and phenolsulphonephthalein tests gave normal findings. An electrocardiogram showed sinus arrythmia; left axis deviation; slurred R waves and flat T waves.

Having established a diagnosis of myxedema and proving from the bone-age studies that its onset was in childhood, she was started on cautious doses of thyroid substance, grains ½ daily.

After a few days this was increased to one grain; and again in the course of a few days to two grains daily. After two weeks on this dosage the basal

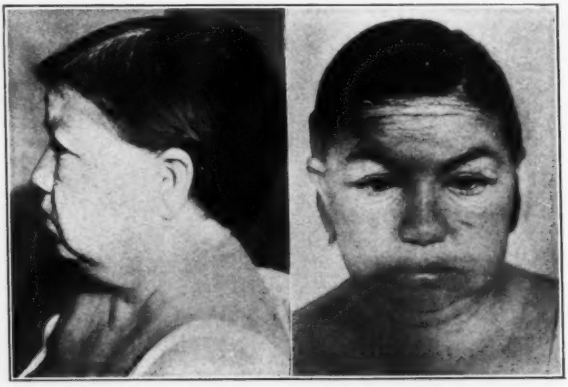


Figure 7. (Case 2). Childhood myxedema in women aged 34 years. Before treatment. Note abundant coarse straight hair, bloated face, pigmentation, stupid expression, coarse wrinkling and puffy eyelids with narrowed palpebral apertures.

Figure 8. (Case 2). Same patient after 10 months treatment with thyroid substance. Puffiness, pigmentation and coarse wrinkling have disappeared. The expression is alert and the scalp hair curly.

metabolic rate had risen to 23.5 per cent minus. Thereupon the thyroid dosage was increased to three grains daily. In another two weeks the metabolism had been raised to 1.4 per cent minus; the patient had lost 13 pounds and the pulse rate had risen to around 100. She still showed some puffiness around the eyes. No tremor nor lid-lag could be elicited, but because the patient had fainted twice on the day preceding this last test, and complained of some inward trembling, it was decided to reduce the dose of thyroid to two grains daily.

Three months after treatment was instituted the patient weighed 85 pounds (a loss of 22 pounds). Her basal metabolic rate was 11.9 per cent plus and her hemoglobin had come up to 77 per cent and the red blood cells now numbered 4,510,000 despite the fact that she had received no medication other than desiccated thyroid.

After six months of treatment her voice was practically normal in pitch and quality. The eyebrows had again filled in and the hair on the scalp had become soft and curly. The puffiness around the eyes completely disappeared and their expression was bright and clear.

Nine months after starting treatment, the patient menstruated for the first time since thyroid had been given. The period was of four days' duration. The flow was normal and free from pain. At this time her height was 4 feet 5 inches (a growth of one inch) and she weighed 75 pounds (a loss of 32 pounds). The hemoglobin was now 90 per cent and red blood cells numbered 5,200,000. The blood serum calcium was 10.6 mg. per 100 cc. An electrocardiogram at this time showed a great improvement in the T waves. X-rays at this time showed that the "bones are aging rapidly and are becoming of the adult type."

One year after thyroid therapy was started this patient menstruated for the second time and her periods have been regular since then, occurring once every 30 to 34 days and lasting four days. Pubic hair has appeared and her skin has become clear and free of the pigmented areas which were present on the face and neck. She is now an alert, cheerful and attractive young woman, utterly transformed. Indeed when comparing the extraordinary photographs of "Before and After," it is difficult to believe that they represent the same individual with a lapse of but ten months' time (See figures 7 and 8).

Less than a year after this patient was started on treatment she became interested in the company of men for the first time. Some nine months later she married and is apparently leading a normal sex life.

(Since the above was written this patient became pregnant, has come to term and will probably be delivered by Cesarean section.)

Summary of Case 2

A woman of 34 who sought medical aid for mere stiffness of the knees only, was recognized as a frank case of childhood myxedema with typical facies, deep-pitched voice, stunted growth, bone-age of 10 years, retarded sexual development with onset of irregular menses at 16 or 17, limited intellectual capacity borne out by a mental rating of 12 years 1 month, and a pronounced secondary anemia.

Under thyroid therapy alone the stiffness of the knees vanished, her facial expression and appearance was radically altered, she grew one inch, her menstrual periods and sex reactions became normal, the anemia cleared up and her mentality improved remarkably.

Comment. This case serves well to call to our attention one of the many and varied symptoms under which hypothyroidism may be veiled. Truly, in this case the diagnosis of childhood myxedema could not be missed, but in the milder degrees of hypothyroidism the chief complaint might be and often is that of vague rheumatic pains. Some years ago Engelbach stressed this point with the dictum that in any case of "rheumatism" which failed to respond to salicylates after three weeks' trial, the patient should be given a basal metabolic test.

Bone-age studies proved conclusively that the onset of her thyroid deficiency antedated adolescence, and, of course, this was suspected clinically on account of her shortness. In spite of markedly retarded bone-age, thyroid therapy merely stimulated the closure of the epiphyses possibly through the indirect stimulation of the gonads, for we were able to produce but one inch of growth in this patient. It is possible that had this patient been treated with a potent anterior pituitary growth fraction, along with thyroid, a more definite gain in stature might have been achieved.

Anemia is a relatively common finding in thyroid deficiencies and the blood picture will sometimes return to normal on thyroid therapy alone.

Mental capacity or intelligence ratings do not always give us a true picture of a patient. In this case the I. Q. showed an advancement of only two years as the result of two years of thyroid therapy. The same doctor performed both tests on this patient and remarked that there was a miraculous change. Her answers in the first test were given with pathological slowness and difficulty, and her responses two years later were made with a certainty and an alertness that was most refreshing.

SUMMARY

1. Two cases of childhood myxedema are reported; one a girl of 12, the other a young woman of 34. The 12-year-old girl showed an ossification age of 6 months to one year—an amazing retardation. In the woman of 34 the epiphyses were still ununited and her bone age was estimated at 10 years.

2. The diagnosis of childhood myxedema was clinically obvious in both patients but roentgenographic studies of bone-age were an important item in gauging the severity of thyroid deficiency.

3. A basal metabolism test could not possibly have been performed in the stupid child of 12. Bone-age studies were easily obtained, with accuracy, and without discomfort.

4. Other ailments, endocrine and non-endocrine, which influence ossification are briefly referred to.

5. Periodic bone-age determinations constitute a simple and helpful adjuvant in judging the efficacy of thyroid therapy in childhood myxemeda. This is excellently exemplified in the first case report.

REFERENCES

1. Gordon, M. B.: Endocrinology, **6**: 235. 1922.
3. Dieterle, T.: Jahrb. f. Kinderh. **64**: 465. 1906. Virchows Arch. f. path. Anat. **184**: 56. 1906. Verhandl. d. Versamml. d. Gesellsch. f. Kinderh. deutsch. Naturf. u. Aerzte. **28**: 276. 1912.
4. Engelbach, W. and A. McMahon: Endocrinology, **8**: 1. 1924.
5. Engelbach, W.: "Endocrine Medicine," Charles C. Thomas, Springfield and Baltimore, 1932.
6. Shelton, E. K.: Endocrinology, **15**: 297. 1931. J. A. M. A. **96**: 759. 1931.
7. Lisser, H.: In "Blumer's Bedside Diagnosis," Vol. 3. p. 72. W. B. Saunders Co., Philadelphia and London. 1928.
8. Lisser, H.: Internat. Clin. **4**: 67. 1933.

THE NATURE OF THE ESTROGENIC SUBSTANCE IN HUMAN MALE URINE AND BULL TESTIS*†

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The fact that male urine and testis tissue contain estrogenic activity has been known for some time. Fee, Marrian, Parkes (1), Glimm and Wadehn (2), Lacqueur and de Jongh (3), Zondek (4, 5), and Loewe (6) have reported the presence of estrogenic substance in male urines. Dodds, Greenwood, and Gallimore (7), Laqueur and de Jongh (3), and Brouha and Simmonet (8) have reported estrogenic activity in testis.

Most of the workers have confined themselves to a qualitative study by means of the reaction on spayed mice or rats. Fee, Marrian, and Parkes have suggested that the active material is identical with the female sex hormone "estrin" on the basis of the marked uterine hypertrophy observed in spayed female rats and the positive vaginal smear reaction. Zondek§ makes the assertion that the estrogenic substance in stallion urine is similar to the follicular hormone on the basis of the production of uterine hypertrophy, an antimasculine action, the induction of lactation in the male guinea pig, and the positive vaginal smear reaction. However, it must be remembered that estrus and uterine hypertrophy are biological reactions produced in greater or less degree by the two female hormones thus far isolated. It has also been shown by Cook, Dodds, Hewett, and Lawson (9) that certain synthetic phenanthrene and dibenzanthracene derivatives are also capable of producing estrus and uterine hypertrophy. Therefore, we feel that a quantitative biological study is necessary to differentiate among theelin, theelol, and any other possible estrogenic substance.

In this communication we have undertaken to characterize this estrogenic activity in human male urine and bull testis by comparative quantitative biological studies on these preparations and on the crystalline female sex hormones, theelin and theelol.

For the urinary preparations, 110 liters of normal male urine were collected from males ranging in age from 20 to 30 years. Immediately after collection, the urine was acidified with HCl to approximately 1 per cent by volume, the urine was extracted four times with benzene in a continuous extractor already described by Gallagher and Koch (10). The

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§After this paper had been presented, Deulofeu and Ferrari (15) reported that they obtained crystalline estrus-producing hormone from stallion urine.

benzene solution was evaporated to dryness, taken up in ethyl ether, and shaken with aqueous sodium bicarbonate solution, and finally with water. The ether solution was evaporated to dryness, yielding a viscous red brown oil which was made up to volume in 95 per cent ethanol. This total benzene extract will be referred to as UD II total extract. A measured portion of the alcohol solution, UD II total extract, was evaporated to dryness, taken up in 200 cc. of ethyl ether, and extracted 25 times with 100 cc. portions of fresh 10 per cent sodium hydroxide solution. The watery alkaline extracts were combined and made acid by addition of an excess of 10 per cent sulphuric acid. The acid solution was extracted with ethyl ether. The ether was distilled and the remaining brown oil was made up to volume in 95 per cent ethanol. This fraction will be known as UD II alkali-soluble. The ether solution containing the alkali insoluble fraction was evaporated to dryness and the reddish oily residue, made up to volume in 95 per cent ethanol, will be referred to as UD II alkali-insoluble.

The theelin assayed 7630 international rat units per mgm. from which it might be inferred that our preparation is slightly inferior to the international standard. The theelol assayed 125 international rat units per mgm., which compares favorably with the values of Butenandt and Browne (16) by essentially the same method of assay as we used, namely, one injection of theelol dissolved in oil.

The theelin and theelol employed in this study were obtained from pregnancy urine and were the crystalline compounds.

The urine fractions, the bull testis preparations, the theelin, and the theelol were assayed on adult spayed female rats according to the method described by D'Amour and Gustavson (11). Twenty-five animals were used for the assay of each preparation and the results referred to a standard which was assayed at the same time. The values given for the estrogenic activity in the charts and graphs are our own rat unit values which are seven times the international rat unit, that is: 1 RU=7 IRU. The male hormone content was determined by the comb growth method published by Gallagher and Koch (12, 13).

Infantile female albino rats from our own stock colony were employed in this study. Injections of the active material in 0.2 cc. of olive oil were started on the 25th day of life and were continued once a day for five days. All values of active substance in the following charts and graphs are expressed in dosage per day. During the injection period the animals were carefully observed each day for vaginal introitus. The animals were killed on the 30th day. Body, ovarian, and uterine weights were determined. Litter mates were used in all of the comparative studies.

Statistical analysis of the data seemed unnecessary in view of the relatively small individual variations and reproducibility of our results. The greatest individual variation encountered occurred in the group of five animals injected with 0.1 RU of theelol where one animal showed a uterine ratio deviating from the mean by 33 per cent. On the average no one

animal in a group of five showed a greater deviation than 20 per cent from the mean uterine ratio, and the majority of animals in the group showed a deviation from the mean of less than 10 per cent. In a group of five animals injected with 0.1 rat unit of theelin we found a mean uterine ratio of 0.751. To test the reproducibility of our results, another group of five animals was injected under the same conditions. This time we recorded a mean uterine ratio of 0.797, giving us a deviation of 5 per cent from the first observation. So also on 0.3 rat unit of theelin, the mean

TABLE I

Estrogenic Activity R. U.	No. of Animals on Each Preparation	Mean Uterine Weights in Mgm.			
		Theelin	Theelol	UD II Alkali Soluble	UD II Total Extract
0.01	5	20.4	35.7	....	20.7
0.10	5	36.8	49.0	43.8*	66.9‡
0.20	5	53.2	40.2	57.3	83.9
0.30	5	82.9	51.7	99.3	123.
0.55	3	206.0	....	....	....
1.0	3	....	85.5	....	....
1.5	3	....	70.5	....	....

*0.12 rat unit. ‡0.11 rat unit. On a given dose the comparison of the four preparations was made on litter mates.

uterine ratios were 1.78 and 1.60 on two groups of five animals each. It is evident, therefore, that our values are significant and reproducible. It is likewise apparent that when four preparations are compared as for example in Tables I, II, III and IV, the significance of the results is increased by the use of a littermate animal on each preparation, as we have done throughout.

TABLE II

Estrogenic Activity R. U.	No. of Animals on Each Preparation	Ratio = Mgm. of Uterus / Gm. of Body Weight Less Gut			
		Theelin	Theelol	UD II Alkali Soluble	UD II Total Extract
0.01	5	0.499	0.862	....	0.519
0.10	5	0.751	1.059	0.893*	1.56‡
0.20	5	0.978	0.819	1.013	1.75
0.30	5	1.78	1.10	2.07	2.53
0.55	3	5.1	....	....	....
1.0	3	....	1.7	....	....
1.5	3	....	1.4	....	....

*0.12 rat unit. ‡0.11 rat unit.

Tables I, II, and III present a summary of the uterine and vaginal responses to various levels of estrogenic activity of the urinary preparations compared to theelin and theelol. In Table I we have the response expressed in mgm. of uterine weight, in Table II, the ratio of mgm. of uterus to gm. of body weight less gut, and in Table III, vaginal introitus in per cent.

TABLE III

Estrogenic Activity R. U.	No. of Animals on Each Preparation	Vaginal Introitus in Per Cent			
		Theelin	Theelol	UD II Alkali Soluble	UD II Total Extract
0.01	5	0	60	....	0
0.10	5	0	100	0*	70‡
0.20	5	30	100	20	100
0.30	5	100	100	100	100
0.55	3	100	....	....	....
1.0	3	....	100	....	....
1.5	3	....	100	....	....

*0.12 rat unit. ‡0.11 rat unit.

We find that in doses of 0.01 rat unit, expressed in terms of estrogenic activity, the estrogenic substance shows a similarity in vaginal introitus and uterine weight reactions to theelin but quite different from theelol. At 0.1 rat unit the estrogenic substance in the total benzene extract (UD II total extract) does not resemble theelin alone, but the alkali-soluble fraction (UD II alkali-soluble) from the total extract closely approximates theelin on both the vaginal introitus and uterine weight reactions. The alkali-insoluble fraction (UD II alkali-insoluble) when injected in a concentration equivalent to the volume of urine represented in 0.1 rat unit of UD II total extract gives no response on the uterus or vagina. When

TABLE IV

UTERINE HYPERTROPHY AND VAGINAL INTROITUS

Responses Produced by Different Levels of the Alkali-Insoluble Fraction from Normal Male Urine Compared to Untreated Normals

Daily Dosage of UD II Alkali-Insoluble		Number of Animals Used	Uterus	Body Weight Less Gut	Ratio: Uterus / Body Weight Less Gut	Vaginal Introitus
Liters of Urine	B. U.		Mgm.	Gm.		Per Cent
0.0	0.0	18	23.4	40.8	0.540	0
0.014	0.75	5	27.4	51.8	0.537	0
0.385	20.6	3	58.1	52.1	1.092	100
1.32	70.6	2	102.6	44.4	2.40	100

UD II alkali-insoluble and UD II alkali-soluble are recombined and administered in identical dosage, they give a response equivalent to UD II total extract on both the uterus and the vagina. From this we postulate a substance present in UD II alkali-insoluble which exerts an enhancing effect on the vagina and uterus when combined with theelin. Increasing the estrogenic level to 0.2 and 0.3 rat unit we find essentially confirmation of our results at the lower levels. At 0.3 rat unit the small discrepancy between theelin and UD II alkali-soluble probably is due to small amounts of the enhancing substance mechanically introduced into the alkali solution during the 25 extractions with 10 per cent sodium hydroxide.

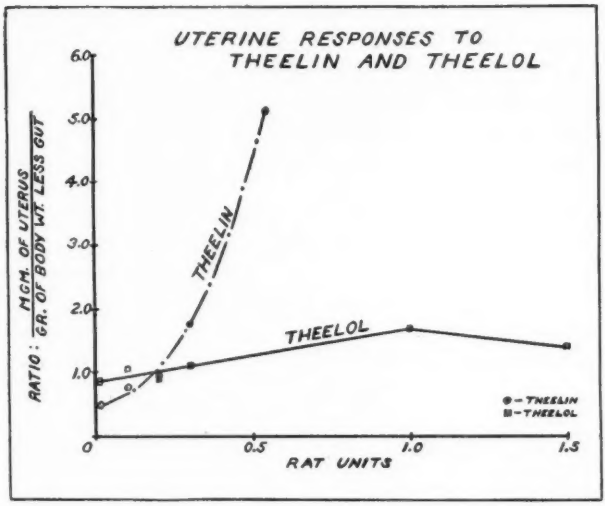

FIGURE 1

In Figure 1 we see the characteristic curves of uterine hypertrophy responses for theelin and theelol. We see at a glance the striking difference between the two female sex hormones. Although theelol gives a positive response on the uterine hypertrophy reaction at a lower rat unit level, theelin is more efficient and produces a more marked effect with increase in estrogenic activity. This peculiar difference in quantitative response to increasing doses of rat units supplied in the form of theelin and theelol obviously gives us a very good biological method by means of which to

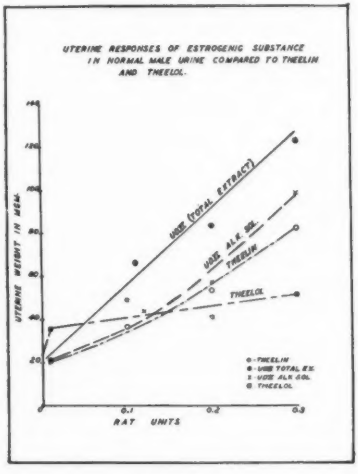

FIGURE 2

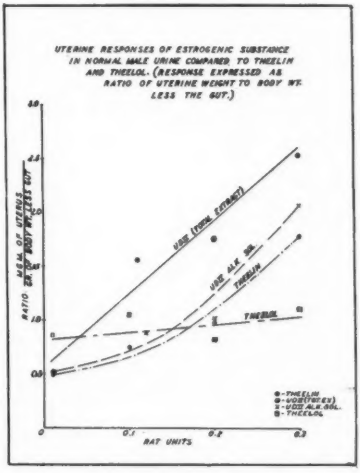

FIGURE 3

distinguish between these two substances in various biological preparations. The data presented in this paper confirm the value of this method of approach.

In Figures 2 and 3 we have composite curves of UD II total extract, UD II alkali-soluble, theelin, and theelol. In Figure 2 uterine hypertrophy is expressed in mgm. while in Figure 3 it is expressed in the ratio of mgm. of uterine weight to gm. of body weight less the gut. These graphs summarize the data in the tables, I, II, and III, and also show that we get the same significant results whether the response is expressed in weight of uterus or in ratio of uterus to body weight. By comparing the curves for UD II alkali-soluble and theelin, we observe a very striking similarity whereas theelol and UD II total extract do not agree with each other and also do not agree with theelin. This clearly shows first, that the

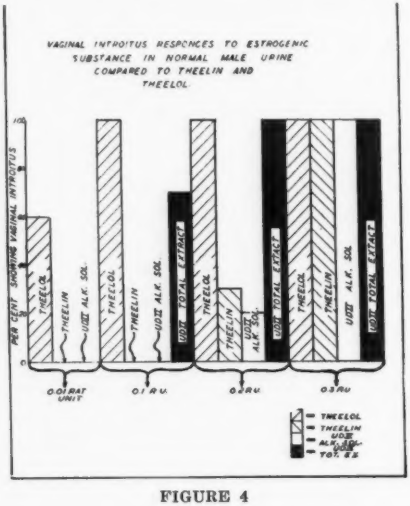

FIGURE 4

estrogenic activity extractable from the urinary male hormone by aqueous sodium hydroxide is theelin and not theelol and, second, that a uterine-enhancing substance other than theelin and theelol is present in the total urinary extract. It is interesting to note that if we compare the vaginal introitus activity of theelin and theelol on a weight basis we find theelin to be the more active. This observation is contrary to that of Curtis and Doisy (14), who, however, employed a different technique than we did.

Figure 4 is a summary of the data dealing with vaginal introitus. It brings out essentially the facts that on a rat-unit basis theelol is more active than theelin, that UD II alkali-soluble is strictly comparable to theelin, and that UD II total extract is more active on the vagina than UD II alkali-soluble.

In view of the marked difference in the uterine hypertrophy and vaginal introitus reactions of the total extract (UD total extract) and the

alkali-soluble extract (UD II alkali-soluble) it became extremely interesting to investigate the alkali-insoluble fraction (UD II alkali-insoluble).

Table IV gives a summary of the responses obtained at three levels of UD II alkali-insoluble compared to uninjected normals of the same age. At a level equivalent to 0.014 liter of urine we see no effect on uterus or vagina, but at 0.385 liter and 1.32 liters of urine we observed a distinct and definite reaction.

The question now arises as to the material in UD II alkali-insoluble responsible for this uterine hyperthophy and vaginal introitus. What are the possibilities? We are led to consider the four following possibilities: first, the remote possibility of theelol present in UD II alkali-insoluble; second, theelin alone; third, male hormone or some undescribed substance; fourth, theelin plus enhancing substance.

At the present time we cannot present a complete solution of this problem but merely indicate our observations which no doubt throw some light on this complicated system. As to the first mentioned possibility we may say that it is eliminated because it is very improbable that theelol, being more soluble in aqueous alkali than theelin, would not be extracted after 25 extractions with 10 per cent sodium hydroxide. Further, by calculations from the theelol curve we find that since the dosage equivalent to 0.385 liter of urine of UD II alkali-insoluble gives us a uterine ratio of 1.09, the highest dosage 1.32 liters of urine should give a uterine ratio of 1.69 if the typical theelol reaction were obtained. However, we actually find 2.40 (see Figure 1). The second possibility is promptly ruled out by similar calculations from the pure theelin curve. From the curve in Figure 1 we should get a ratio above 5 for the highest dilution where we find 2.4. The third possibility, that the action is due to male hormone or some undescribed substance, is under investigation at the present time. It is, of course, possible that the male hormone may be the enhancing substance, thus presenting a possible physiological rôle for the male hormone found in the urine of women. This would parallel the observation of Freud (17) that estrogenic substances play an important part in the response of the mammalian accessory reproductive organs to the male hormone. The fourth possibility, that is, theelin plus enhancing substance, has so far given us some encouraging results. We have tested our values on the UD II total extract curve since this curve represents theelin plus enhancing substance. At 0.385 liter of urine we find a uterine ratio of 1.09 which corresponds to 0.08 rat unit of the UD II total extract. At 1.32 liters, which is 3.4 times the smaller concentration, we should expect the effect from 0.27 rat unit or a uterine ratio of 2.38 (see Table IV and Figure 1). We actually find 2.40, but since the values have been determined on a limited number of animals, too much significance should not be placed on the values.

By a method similar to that employed for the work outlined above on the urine preparations, it has been found by us that the alkali-soluble frac-

tion from the total extract of bull testis contains an estrogenic substance similar to theelin on the basis of vaginal introitus and uterine hypertrophy.

SUMMARY

We have compared the uterine hypertrophy and vaginal introitus responses of equivalent rat unit dosages of theelin, theelol, and estrogenic substance from male urine. These observations were made at estrogenic levels ranging from 0.01 rat unit to 0.3 rat unit. Our method involved the daily administration of the active material in olive oil to 25-day-old female albino rats for five days, and observing the uterus and vagina on the sixth day. At each rat unit level for each preparation five animals were used except in a few cases where three animals were deemed sufficient. Litter mates were used throughout the comparative studies. A total of 161 animals were used for the comparative studies.

The quantitative data presented lead to the following conclusions:

1. There is a distinct difference between theelin and theelol in their respective abilities to cause hypertrophy of the uterus and cause vaginal introitus. On an estrus-rat-unit basis, theelol is more effective in causing vaginal introitus as originally demonstrated by Curtis and Doisy (14), whereas theelin produces an enormously greater uterine hypertrophy.

2. The estrogenic substance in the total benzene extract from normal male urine resembles neither theelin nor theelol in causing either vaginal introitus or uterine hypertrophy.

3. The estrogenic substance in the alkali-soluble fraction of the total benzene extract is biologically identical with theelin. It is not comparable to theelol in causing either vaginal introitus or uterine hypertrophy.

4. The evidence presented points to the presence of a substance in the alkali-insoluble fraction of the total extract which, when administered with theelin, enhances the action on both the vagina and the uterus. This substance is neither theelin nor theelol.

REFERENCES

1. Fee, A. R., G. F. Marrian and A. S. Parkes: The significance of the occurance of oestrin in male urine, J. Physiol. **67**: 377. 1929.
2. Glimm, E. and F. Wadehn: Über das Vorkommen von weiblichen Sexualhormon in Harn, Biochem. Z. **243**: 97. 1931-32.
3. Laqueur, E. and S. E. de Jongh: A female (sexual) hormone, J. A. M. A. **91**: 1169. 1928.
4. Zondek, B.: Mass excretion of oestrogenic hormone in the urine of the stallion, Nature, **133**: 209. 1934.
5. Zondek, B.: Oestrogenic hormone in the urine of the stallion, Nature, **133**: 494. 1934.
6. Loewe, S., H. E. Voss, F. Lunge and A. Wöhner: Sexualhormonbefunde in männlichen Harn, Klin. Wchnschr. **7**: 1376. 1928.
7. Dodds, E. C., A. W. Greenwood, E. J. Gallimore: Note on a water-soluble active principle isolated from the mammalian testis and urine and its relation to oestrin, Lancet, **228**: 683. 1930.
8. Brouha, L. and H. Simmonet: Action d'extracts orchitiques liposolubles sur le tractus génital femelle, Compt. rend. Soc. de biol. **99**: 41. 1928.
9. Cook, J. W., E. C. Dodds, C. L. Hewett and W. Lawson: Estrogenic activity of some condensed ring compounds in relation to their other biological activities, Proc. Roy. Soc., London, S. B. **114**: 272. 1934.
10. Gallagher, T. F. and F. C. Koch: Biochemical studies on the male hormone as obtained from urine, Endocrinology, **17**: 107. 1934.

11. D'Amour, F. E. and R. G. Gustavson: A critical study of the assay of female sex hormone preparations, J. Pharmacol. & Exper. Therap. **40**: 473. 1930.
12. Gallagher, T. F. and F. C. Koch: The quantitative assay for the testicular hormone by the comb growth reaction. J. Pharmacol. & Exper. Therap **40**: 327. 1930.
13. Unpublished data.
14. Curtis, J. M. and E. A. Doisy: The bioassay of theelol. J. Biol. Chem. **91**: 647. 1931.
15. Deulofeu, V. and J. Ferrari: Crystalline oestrus-producing hormone from horse (stallion) urine. Nature, **133**: 835. 1934.
16. Butenandt, A. and J. S. L. Browne: Vergleichende Untersuchung von Theelol, Emmenen, und Follikelhormonhydrat. Ztschr. f. physiol. Chem. **216**: 49. 1933.
17. Freud, J.: Conditions of hypertrophy of the seminal vesicles in rats. Biochem. J. **27**: 1438. 1933.

ON THE COMPARATIVE LUTEINIZING CAPACITY OF THE URINE OF PREGNANCY AND OF THE MENOPAUSE

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Recently new interest has arisen concerning the question of the gonadotropic hormones in the urine of pregnancy and of the menopause since it has been shown by Engle (1), and later by many investigators, that the gonadotropic action of these urines is not identical to that of the anterior lobe of the hypophysis. This comparative interest is all the more justified since we know that the gonadotropic action of certain hypophyseal extracts is modified by simultaneous administration of urine. There is in the urine of pregnancy (Leonard, 2; Evans and associates, 3) and in the urine of the menopause (Lipschütz, 4) a gonadotropic substance which is neither the oestrogenic, nor the luteinizing one. The exact position of this third substance in the anterior gonadotropic complex is yet to be determined. Leonard (17) also suggested the possibility of there existing a separate ovulating principle, or of a fourth gonadotropic hormone according to the above scheme.

According to the statement of Zondek (5) the urine of the menopause differs from that of pregnancy in that the first has an oestrogenic action but not a luteinizing one. The oestrogenic activity of the urine of the menopause is in general many times less intense than that of the urine of pregnancy. So one might suppose that possibly the urine of the menopause, as far as luteinization is concerned, differs from that of pregnancy only quantitatively, all the three gonadotropic factors being present in both urines. The urine of the menopause then would represent as far as the gonadotropic hormones are concerned, diluted urine of pregnancy.

In the course of work on the third gonadotropic hormone in the urine of the menopause (Lipschütz, 6) we stated that this urine reveals also a luteinizing capacity if sufficient quantities of urine are injected into the infantile female rat. A similar statement was recently made by Oesterreicher (7) in work on the mouse. Working with Jara (8) in this laboratory, we found the same to be true when injecting great quantities of urine of the menopause into adult rabbits. So we decided to examine comparatively the luteinizing action of the urine of pregnancy and of the menopause, making use of the method the writer (Lipschütz, 8) has devised for determining the luteinizing capacity of the hypophysis.

METHODS

The urine of pregnancy was given by subcutaneous injections repeated six times in less than two days; the hormones were not precipitated but urine as such was given. Infantile female rats are able to survive even when more than 10 cc.

of urine are injected in the course of two days. Extracts of the urine of the menopause were prepared by the routine method devised by Zondek (5), i.e., precipitating the urine with alcohol, washing the precipitate with ether and extracting with Ringer's solution. In this manner equivalent quantities of up to 280 cc. of urine can be injected into the infantile female rat in 1 to 2 days.

The method for the quantitative determination of the luteinizing capacity of the urine was identical with that for the determination of the coefficient of luteinization or Qlut of the hypophysis which has been described elsewhere in detail (Lipschütz, 10, 6). It might be repeated here that every tenth microscopical slide of the ovary is projected on white paper and the outlines of the ovary and those of the corpora lutea are drawn with pencils of different colors, the whole drawings and the corpora lutea, respectively, are cut out and weighed. The percentage of luteinization in the ovary is easily calculated; knowing the weights of the ovaries the quantity of the luteal tissue produced also is calculated. As to the hypophysis Qlut is equal to the quantity of luteal tissue produced divided by the quantity of hypophysis administered. As to the experiments with urine, the quantity of luteal tissue produced in milligrams is divided by the quantity of urine in cubic centimeters and multiplied by 1000, the luteinizing faculty of one liter of urine being thus determined in milligrams. Calculations might be simplified by equations we have given elsewhere (Lipschütz, 6). For the present experiments with urine the equation is:

$$\text{Qlut. urine} = \frac{l}{n} .1000 = \frac{o \times w \times 1000}{W \times n}$$

l being the quantity of luteal tissue produced in milligrams, n the quantity of urine injected in cc., W the weight of the cuttings of the whole ovary, w the weight of the cuttings of the corpora lutea, and o being the weight of the ovary.

We always calculated first the percentage of luteinization $\frac{(w \times 100)}{W}$, the results becoming thus much more instructive.

EXPERIMENTAL RESULTS

A. Urine of Pregnancy. In Table 1 the data of experiments with two different urines of pregnancy are shown. Animals with the same Roman numbers belong to the same litter.

As seen from the upper half of the table, 2.4 cc. of urine might produce more than 2.5 to 6 mg. of luteal tissue. Although there are considerable variations between two animals of the same litter receiving the same quantity of urine (Exp. 1 and 6, 2 and 5), the quantity of luteal tissue produced in general increases with the quantity of urine administered. The same is seen when animals of the same litter are compared in the lower half of the table: III/1 and III/2; IV/1, IV/2 and IV/3; V/1 and V/2. As little as 1.2 cc. of urine might produce 11 mg. of luteal tissue, but the quantity of luteal tissue produced is by no means proportional to the quantity of urine given. On the contrary, the coefficient of luteinization as calculated for 1 liter of urine diminishes when greater quantities of urine are given.

A lack of proportionality between the quantity of urine given and the intensity of gonadotropic action as judged by ovarian weights, has already been observed by Evans, et al. (2), Emery (14) and Fluhmann (15). Our experiments with the determination of Qlut give further corroboration.

One must assume that a factor is acting which limits luteinization and opposes proportionality. One conception is that the limiting factor is in

TABLE 1

No.	Age of Animal Days	Weight at First Injection gm.	Weight When Killed gm.	Killed After First Injection Days	Urine Injected cc.	Weight of Ovary mg.	Per Cent of Lutein-ization	Lutein Tissue Produced mg.	Qlut. (Lut. Tissue Produced Per Liter in mg.)	Qlut. Average	
I/1	35	35	43	6	2.4	13	21.3	2.76	1150	1855	Urine of the fifth month of pregnancy. Six injections during 42 hours.
I/6	35	38	48		2.4	18	34.2	6.15	2560		
I/2	35	33	40	6	4.8	7	42.2	8.89	1850	1330	
						11	54.0				
I/5	35	38	43	6	4.8	12	32.6	3.89	810		
I/4	35	33	38	6	9.6	15	48.9	7.35	765	860	
I/7	35	38	44	6	9.6	5	32.9	9.19	955		
						13	58.0				
IV/1	26	27	40	7	1.2	8	46.9	9.37**	7800	8565	Urine of the sixth month of pregnancy; same urine in all litters. Six injections during 41 to 42 hours.
						10	56.2				
V/1	26	29	44	7	1.2	10	43.3	11.2†	9330		
						13	52.8				
IV/2	26	27	41	7	2.4	20	58.0	11.6**	830	6130	
V/2	26	29	45	7	2.4	15	58.4	17.9†	7430		
						17	53.3				
III/1	35	45	50	6	3.6	26	49.0	12.7*	3540	3540	
IV/3	26	27	36	7	4.8	10	52.5	13.6**	2840	2840	
						14	59.6				
III/2	35	45	56	6	10.8	31	57.9	17.9*	1660	1660	

*, **, † of same litter, respectively.

the ovary itself, the ovary of the infantile female rat being able to furnish only a limited number of follicles capable of being luteinized at the given moment. Now when the percentage of luteinization in the ovaries of animals receiving great quantities of urine is examined, it is found that this assumption cannot hold. With the urine of pregnancy the percentage of luteinization was always less than 60 even when as much as 10.8 cc. of urine was injected, the figures varying between 21 and 60 per cent. On the contrary, with the anterior hypophysis the writer (6) found that the luteinization of the ovary of the infantile rat may attain as much as 83 per cent. This is indicative that the ovary of the infantile rat can be luteinized to a much higher degree than was found in the above experiments with urine. Fluhmann (18) has also recently shown that when the injection of an extract of pregnancy blood is continued for 10 to 15 or 20 days, a much greater increase in ovarian weight can be obtained in the immature rat than when the same total dose is given in five days. It might then seem that the limiting factor is to be found simply in the timing conditions of the experiments, the hormones introduced with the urine in the course of 40 to 42 hours being soon destroyed or eliminated. In experiments with the rabbit it can indeed be easily demonstrated that the gonadotropic principle of the urine introduced by intravenous injection rapidly disappears from the blood.

On the contrary, with the hypophysis a fair degree of proportionality is found in the infantile female rat, as shown by Evans and associates (1) and by Fluhmann (15), by weighing the ovary and by the writer (10) by determining Qlut. Neither can the limiting factor with urine of pregnancy be some inhibiting substance since Emery (16) has shown that urine will not prevent the anterior pituitary sex hormone from causing enlargement of the ovaries. (See also the above mentioned work of Leonard, Evans, Fevold, Lipschütz with combination of hypophysis with urine.)

As seen from experiment IV/1, 8000 to 9000 mg. of luteal tissue can be produced by 1 liter of urine of pregnancy. It is of considerable interest to compare these quantitative findings with the coefficient of luteinization of the hypophysis. It has been stated by the writer (10) that Qlut of the hypophysis of the infantile female rat

$$\text{Qlut} = \frac{\text{Mg. luteal tissue produced}}{\text{Mg. anterior lobe administered}}$$

which is highly active is between 1.2 and 4.0, the average in 6 experiments being Qlut=2.56. Qlut of the hypophysis of the adult male rat is less, but ranges next to that of the infantile female. We have determined Qlut in 15 experiments with the administration of the hypophysis of adult male rats as shown in Table 2. The hypophysis was triturated with Ringer and injected subcutaneously.

TABLE 2

QLUT IN ADULT RATS

No.	Body Weight at 1st Injection gms.	Anterior Pituitary Substance Injected mg.	Killed Days After 1st Injection	Qlut	Remarks
1	31	13.5	4	0.03	Animals 3 to 5 weeks old. Every tenth micr. slide drawn, with exception of those with * where only part of the serial slides had been kept and used—determination of Qlut being less exact in the experiments with *.
2	27	13.4	7	0.07	
3*	22	37.	<4	0.09	
4	32	14.5	6	0.46	
5	25	17.	5	0.67	
6*	27	15.5	5	0.72	
7*	26	15.5	5	0.78	
8	32	15.	6	0.83	
9*	23	21.	6	0.85	
10	30	13.5	7	0.95	
11	27	21.	6	0.98	
12*	26	21.	6	0.99	
13*	33	17.2	5	1.03	
14	27	22.	5	1.34	
15*	27	21.	6	1.54	

There are very great variations as to Qlut of the hypophysis of the adult male rat. The data on Nos. 1 and 3 have to be discarded, since in 4 days no luteinization at all might be found. The only experiment in which Qlut is outside the range of the remaining 12 experiments is No. 2. The average of experiments Nos. 4 to 15 is Qlut=1.0.

Thus 1 liter of urine from the middle of pregnancy is equal as far as its luteinizing capacity is concerned, to more than 8,000 to 9,000 mg. of male rat anterior hypophysis. Since the hypophysis of the adult male rat weighs roughly about 5 mg. the daily urine of the pregnant woman may contain as much of the luteinizing hormone as 1600 to 1800 adult male rat hypophyses. It will be of interest to determine in comparative manner the quantitative relation between the luteinizing capacity of the human hypophysis and the urine of pregnancy.

B. Urine of the Menopause. Six samples of urine of climacteric women 48 to 58 years old, from 6 months to 18 years in the menopause, were examined in 18 experiments with infantile female rats. As already related extracts were used. The injections were made in less than 2 days, as with the urine of pregnancy; 1 cc. of extract was equal to 15 to 46 cc. of urine. The results are summarized in Table 3.

TABLE 3

No.	Age of Woman Years	Amenorrhoic Since	Quality of Urine Injected cc.	Oestrous	Qlut (Lut. tissue produced per liter in mg.)	Qlut (Average)
1	50	6 months	80	+	1.4–2.6	2.0
2	50	9 months	150	+	9.5	9.5
3	48	2 years	230	+	18.6	18.6
4	48	2½ years	70–280	+	72.0–177.1	99.7
5	56	10 years	75–140	+	0	0.
6	58	18 years	64–175	+	5.9–8.6	7.4

There was only one woman (No. 5) whose urine did not reveal the luteinizing action when 140 cc. of urine was injected.

These experiments make it very probable that almost every climacteric woman whose urine has an oestrogenic gonadotropic action is eliminating also the luteinizing hormone. As to this there seems to be no difference between the hormone content of the urine of pregnancy and of menopause. But when the coefficient of luteinization is calculated for 1 liter of urine, the difference between the urine of pregnancy and of menopause becomes enormous. In only one case (No. 4) Qlut was of 100 or more mg. per liter of urine of the menopause; in four other cases the coefficient was only 2 to 19 mg. per liter. It becomes evident from these results that the luteinizing capacity of the urine of the menopause is extremely small as compared with that of the urine of pregnancy, the ratio being at least as small as 1:100 or 1:1000.

It might be questioned whether the difference between both urines as far as luteinizing is concerned is of the same quantitative order as the difference between the oestrogenic faculty of both. We have not examined quantitatively this question but it is seen from the work of Zondek and others that the oestrogenic faculty of the urine of pregnancy is probably hundreds of times greater than that of the urine of the menopause.

Jara (8) examined also various samples of urine of normal women. All the five subjects examined (15 to 23 years old, 7 to 8 days after menstruation) were unable to produce luteinization even when an extract of as much as 140 cc. of urine was injected. Oestrous as revealed by vaginal smear was produced only in one case.

DISCUSSION

As seen from the above experiments the urine of menopause is capable of producing luteinization in the rat like the urine of pregnancy, but on a different quantitative level. Does this mean that the urine of menopause contains many times less of the luteinizing hormone or prolan B? It might seem at first sight extraordinary to question this, since one is always identifying less luteinizing faculty with less luteinizing hormone. But it has been shown that as far as the hypophysis is concerned lack of luteinizing capacity does not yet mean lack of luteinizing hormone; the hypophysis of the adult female rat incapable of producing any considerable luteinization in the infantile female rat causes luteinization when injected into the adult female rabbit, and it is the same with the hypophysis of the guinea pig (Lipschütz, Reyes and Viñals, 11).

This differential reaction of the infantile female rat and the adult rabbit might be explained in two different ways. Assuming that the hypophysis of the adult rabbit liberates a third prehypophyseal gonadotropic factor which is necessary for the full ovarian reaction, including luteinization, a gonadotropic factor which though present in is not liberated by the hypophysis of the infantile female rat; or secondly, assuming that the

sensitivity of the adult rabbit to the gonadotropic hormone is greater than that of the infantile female rat, as supposed especially by Aron (12). Now, if this latter explanation were true, then the greater sensitivity as far as luteinization is concerned should reveal itself in the adult rabbit not only with the hypophysis of the adult female rat or the guinea pig, but also with the urine of the menopause. Jara (8) has given injections of considerable quantities of urine of women in menopause or castrated into adult rabbits; extracts equivalent to 200 to 300 cc. of urine were given intravenously, the animals being killed three days afterwards. There was luteinization only in two of six cases (one belonging to a woman 45 years old castrated three weeks previously, the other a woman of 48 with amenorrea for two years and a half). So one might conclude that there is a close parallelism between the very feeble luteinizing capacity of the urine of menopause in the infantile rat and that in the adult rabbit, contrary to the differential reaction of both with hypophysis injections. This is indicative that the very feeble luteinizing faculty of the urine of menopause is really due to the fact that this urine contains very small quantities of the luteinizing hormone, and that the differential reaction to hypophyseal injection is not a matter of greater "sensitivity," but that there is in the adult rabbit some gonadotropic factor at work which is not effective in the infantile female rat. In favor of this assumption are also the experiments of the writer in which the non-luteinizing (or feebly luteinizing) hypophysis of the guinea pig was administered simultaneously with urine of menopause in the infantile rat (Lipschütz 6): very considerable luteinization occurred even then when both sources separately gave no luteinization at all in the controls. Recently Selye, Collip and Thomson (13) came to the conclusion that species differences in the reaction to the urine of pregnancy may be due to differences in the ability of the hypophysis to furnish some complementary substance necessary for the full ovarian reaction; our statements as to the differences between the adult rabbit and the infantile rat in the reaction to hypophyseal injections (Lipschütz, Reyes and Viñals, 11) are in strict agreement with the conclusions of Selye, Collip and Thomson.

SUMMARY

A comparative study of the luteinizing faculty of the urine of pregnancy and of that of menopause was made by determining the quantity of luteal tissue produced by a given quantity of urine injected into the infantile female rat (coefficient of luteinization or Qlut).

When small quantities of urine of pregnancy were injected as much as 8 to 9 mg. of luteal tissue was produced by 1 cc. of urine.

With greater quantities of urine the quantity of luteal tissue was increased, but not in linear proportion to the quantity of urine. Thus, with greater quantities of urine Qlut as calculated per liter tended to diminish.

This lack of linear proportionality is not due to an intrinsic ovarian factor.

The luteinizing capacity of 1 liter of urine of pregnancy with Qlut= 8000 to 9000, is equal to that of about 8000 to 9000 mg. of the anterior lobe of the hypophysis of the male rat with Qlut=1.0.

Almost every sample of urine of menopause (5 of 6 women, 6 months to 18 years in menopause) will produce luteinization when sufficient quantities of urine are administered to the infantile rat.

The coefficient of luteinization as calculated for 1 liter of urine of the menopause varied between 2 and 100.

Since the oestrogenic gonadotropic faculty of the urine of menopause also is only a small fraction of that of the urine of pregnancy, the urine of menopause may be considered as far as the gonadotropic substances are concerned, as a highly diluted urine of pregnancy.*

When urine of menopause is injected into the adult rabbit, no special sensitivity for luteinizing is revealed, as compared with the infantile rat, contrary to what is observed with the hypophysis of certain donors (guinea pig or adult female rat) which cause a differential reaction in the adult rabbit and in the infantile rat, respectively.

The bearing or the lack of a differential reaction with urine of menopause in the adult rabbit and infantile rat is discussed in relation to the question of a third hormone in the gonadotropic complex.

REFERENCES

1. Engle, E. T.: Ovarian responses; differences elicited by treatment with urine from pregnant women and by freshly implanted anterior lobe. J. A. M. A. **93:** 276. 1929.
2. Leonard, S. L.: Increased stimulation of immature rat ovaries by combined injections of prolan and hypophyseal sex hormones. Proc. Soc. Exper. Biol. & Med. **30:** 403. 1932.
3. Evans, H. M., K. Meyer and M. E. Simpson: Relation of prolan to the anterior hypophyseal hormones. Am. J. Physiol. **100:** 141. 1932.
4. Lipschütz, A.: Sur un noveau facteur gonadotrope dans l'urine de la femme en ménopause. Compt. rend. Soc. de biol. **114:** 430. 1933.
5. Zondek, B.: Die Hormone des Ovariums und des Hypophysenvorderlappens. Springer, Berlin. 1931.
6. Lipschütz, A.: Experimental developments on the gonadotropic complex of the anterior lobe of the hypophysis. Quart. J. exp. Physiol. 1934.
7. Oesterreicher, W.: Die Hypophysenvorderlappenhormone bei ausgefallener Keimdrüsenfunktion. II. Mitteil. Klin. Wchnschr. **12:** 896. 1932.
8. Jara, H.: Estudio experimental sobre la accion luteinizante de la orina climatérica. Tesis Inst. Fisiol. Univ. Conc. (Chile), No. 9. 1933.
9. Lipschütz, A.: Nouvelles recherches sur le coéfficient de lutéinisation de la préhypophyse. Compt. rend. Soc. de biol. **112:** 1145. 1933.
10. Lipschütz, A.: Über den Luteinisierungskoeffizienten des Vorderlappens der Hypophyse. Endokrinologie, **12:** 90. 1933.
11. Lipschütz, A., G. Reyes and E. Viñals: Nouveaux faits relatifs à l'action lutéinisante de la préhypophyse du Cobaye. Compt. rend. Soc. de biol. **111:** 852. 1932.
12. Aron, M.: L'hormone préhypophysaire excito-sécrétrice des glandes endocrines génitales (gonado-stimuline). Arch. d'anat. d'histol. et d'embryol. **15:** 329. 1932-33.
13. Selye, H., J. B. Collip and D. L. Thomson: Further studies on production of thecal luteinization by means of A.P.L. (anterior-pituitary-like hormones). Proc. Soc. Exper. Biol. & Med. **30:** 780. 1933.
14. Emery, F. E.: Some chronic effects of the anterior pituitary sex hormone on the weights of body, ovaries, uterus, pituitary and adrenal glands. Endocrinology, **17:** 64. 1933.

*Leonard and Smith (19) have recently found that when urine of pregnancy is combined with urine of menopause, the so-called "augmentation" effect is produced. This statement does not support the above conception of urine of menopause being a highly diluted urine of pregnancy.

15. Fluhmann, C. F.: Compartive studies of gonad-stimulating hormones. Endocrinology, **17**: 550. 1933.

16. Emery, F. E.: Am. J. Physiol. **101**: 246. 1932. (Quot. from 14.)

17. Leonard, S. L.: The nature of the substance causing ovulation in the rabbit. Am. J. Physiol. **98**: 406. 1931.

18. Fluhmann, C. F.: Comparative studies of gonad-stimulating hormones. II. Proc. Soc. Exper Biol. & Med. **30**: 1014. 1933.

19. Leonard, S. L. and P. E. Smith: The hypophyseal-like qualities of the gonadotropic principle found in the urine of certain individuals. Am. J. Physiol. **108**: 22. 1934.

CASTRATION ATROPHY

A CHRONOLOGICAL STUDY OF UTERINE CHANGES FOLLOWING BILATERAL OVARIECTOMY IN THE ALBINO RAT

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Atrophy of the uterus associated with degeneration or absence of the ovaries has been seen and described repeatedly. Observations have been made in a few human cases as well as in laboratory animals.

Kleinwachter (1) summarized cases in the literature, as well as those from his own practice. In this series there was usually a history of amenorrhea following childbirth. On examination, atrophy of the uterus and usually of the ovaries also, was found. The uterus and its cavity were described as definitely smaller than normal.

Eckardt (2) reported one case in which the menses ceased, following an operation. Twenty-two months later the uterus was removed. He found the uterus and its lumen small, mucosa and musculature pale, mucosa thin with epithelium resting in places on musclulature, epithelium cubical, and the gland lumina very small or dilated into cysts.

Workers in the field of ovarian hormones have incidentally pictured, or briefly described, the atrophic uterus associated with bilateral ovariectomy. Allen (3), et al., described the uterus of the mouse and rat as small and anemic with a slitlike lumen. They further stated that there were leucocytes in the epithelium, and that the gland tubes were collapsed. Allen (4) and Hisaw and Leonard (5) by low power photographs showed the smaller size of the atrophic uterus in the rabbit. Allen (6) described the uterus of the monkey as smaller, with thin endometrium, with low epithelium embryonic in type, and as lacking in mitoses in surface and gland epithelium.

Experimental removal of the ovaries and study of the resultant effect on the uterus have been done on the guinea pig, rabbit, and albino rat.

Halban (7) described the atrophic uterus of the guinea pig as follows: uterus as small as newborn, with horns slender threads; deficient development of muscular layers, and especially of mucous membrane, latter smooth not folded; scanty glands; epithelium low and without cilia; and degeneration of musculature, not fatty.

The rabbit has been investigated by Krukenberg (1891) and reported by Eckardt (2), by Buys and Vandervelde (8), by Carmichael and Marshall (9), and by Shattock (10). Krukenberg killed his rabbits 9 to 10 months after operation, and found a decrease in weight of the uterus with fatty degeneration and atrophy of the ciliated epithelium. Buys and Van-

dervelde used five rabbits. At operation they removed one uterine horn as a control. The rabbits were killed at 10, 20, 30, 50 and 67 days, respectively, after operation. The atrophic changes appeared at 20 days and were as follows: simple atrophy of the muscle, subepithelial fibrous mass, progressive thickening of the arterial walls, and necrosis of gland epithelium with cyst formation produced by fibrous change in subepithelium. Carmichael and Marshall reported on five adult rabbits killed 3½ to 6½ months after ovariectomy. They found fibrous degeneration of the uterus, most marked in the mucous membrane. In two rabbits castrated before they had reached maturity and killed 8 and 10 months later, they found the uterus infantile in type. Shattock measured the uterus grossly and showed its smaller size. He described it as thin-walled and flaccid.

Marshall and Jolly (11), investigating atrophy in the albino rat, used 33 rats which were 2½ to 8 months post-operative. They described the changes in the uterus as follows: small and atrophic, mucosa thin and poor in cells, not infrequently fibrous overgrowth of mucosa, glands small and sometimes hardly recognizable, and muscular wall thinned.

EXPERIMENTAL

In carrying on this investigation, the authors had the following objectives in view: to ascertain how early definite atrophy occurs; to locate its site; to measure its degree; to follow its progress chronologically; and to describe the histological changes seen in the several coats of the uterine wall.

All castrations were performed in stage V, or interval, of the cycle and all controls were taken in this stage. The descriptions of the stages by Long and Evans (12, 13) were followed in the examination of the vaginal smears. The method of obtaining the smears was modified. In place of speculum and swab, a medicine dropper was used to introduce a small amount of psysiological salt solution into the vagina. This solution was withdrawn into the dropper, placed on a glass slide and dried. The smear was stained with hematoxylin and eosin and examined under oil immersion magnification.

The rats used in this experiment were of Wistar stock, and were free from disease and previous experimentation. The operations were performed with strict aseptic technic, under ether anaesthesia by cone. Through a mid-ventral incision about one-half inch in length, the uterine horns and ovaries were delivered alternately to the surface and received on a sterile pad. In each case, the horn was clamped and the ovaries and tubes removed by excision. When bleeding had been controlled the horns were returned to the abdominal cavity and the incision closed with silk sutures. The animals were then placed in wire cages with raised screen floors. In all cases healing was rapid and complete.

In conducting these experiments, it was originally intended to use a litter mate as a control for each castrated animal, thus assuring identical age and approximately the same body weight for each castrate and control.

It was found, however, that uterine measurements, even in litter mates, varied too much for accurate comparisons on this basis. In order to reduce this variable to a minimum, it was decided to use the average measurements of a number of normal uterine horns for controls. On this basis, 14 rats were castrated and, after subjecting them to the conditions of the experiment described below, their respective atrophic horns were compared with the averages of 30 normal horns. The data resulting from this series are presented in Table II.

Finally, in order to insure that all possible variables, i.e., age, general body weight and cyclic phase, were entirely eliminated, a second series of 18 rats were castrated in stage V, or interval. In this series, one uterine horn was removed along with the ovaries. This horn, in each case, was carried as an individual control for each castrate. In situ measurements with a micrometer caliper at the time of operation and autopsy, respectively, showed that, although there was considerable variation in the several horns, from the bifurcation to the tubal extremity, corresponding portions of the two horns varied little, if any. Therefore, this mid-portion was selected for gross comparisons and sections.

Following the bi-lateral ovariectomy, the rats were autopsied at regular intervals and a careful search was made for ovarian tissue. In a few cases fragments of such tissue were found and these animals were discarded. In addition, all castrated rats were subjected to a series of vaginal examinations and each showed a stage of permanent interval, or stage V.

The uterus and a portion of the vagina of each rat were removed and placed in 10 per cent formalin. After fixation for 48 hours a block of tissue was taken from each horn, mid-way between the bifurcation and the tubal extremity. In the normal horn, this was found to be about 1 centimeter from the bifurcation and in the atrophic horn about 0.5 centimeter. The block was then dehydrated, sectioned in paraffine and stained with hematoxylin and eosin. The same procedure was followed in both control and atrophic horns. In mounting, sections were taken at arbitrary intervals from the entire block of tissue and these were used for both micro-measurements and histological study.

The data obtained, and here reported, were of two general classes: dimensional or quantitative, and histological or qualitative. The former were obtained by actual measurements (under low power magnification of the microscope) of the endometrium, peri-myometrium, total thickness of the uterine wall, the lumen, and the total diameter of the uterine horn, including the lumen. The peri-myometrium proved to be essentially myometrium since the perimetrium remained a very thin membrane.

In obtaining the statistical data, measurements were made in each quadrant of the respective cross sections on lines perpendicular to each other and passing through the longest and shortest diameters, respectively, of the sections. The figures derived from the measurements of all four quadrants were averaged for the expressed thickness of each coat of the

uterine wall and for the average diameter of the wall. The tabulated diameters of the lumen and total horn represent the averages of the two transverse diameters of these areas. In Table II, the figures represent the averages from only one section of each born, while in Table III, they represent the averages from five sections of each horn, or a total of 20 measured diameters of the respective uterine coats and 10 diameters of the lumen and total uterus, respectively. In the tables, these measurements are expressed in millimeters or fractions thereof. Percentages were computed from these figures. The micrometer caliper readings were not tabulated.

TABLE I

MEASUREMENTS* OF NORMAL UTERINE HORNS

Rat No.	Age (days)	Weight (gms.)	Endometrium	Myometrium	Total Wall	Lumen	Total Uterus
62	87	....	.360	.270	.631	.483	1.740
93	98	171	.408	.328	.736	.574	2.046
95	98	148	.608	.356	.964	.639	2.567
79	103	195	.572	.367	.939	.611	2.490
84	103	197	.557	.423	.980	.634	2.595
85	103	184	.585	.456	1.041	.836	2.918
89	104	173	.493	.344	.838	.495	2.172
90	104	186	.354	.356	.711	.518	1.939
91	104	185	.608	.348	.956	.754	2.656
92	104	185	.392	.359	.751	.344	1.846
97	104	185	.405	.380	.785	.552	2.126
98	104	188	.528	.334	.862	.438	2.162
82	105	179	.661	.393	1.054	.615	2.725
77	113	163	.467	.403	.870	.833	2.562
83	113	186	.413	.328	.741	.639	2.118
86	113	184	.537	.344	.851	.685	2.392
94	133	185	.603	.290	.893	.666	2.452
96	133	211	.528	.287	.815	.521	2.152
24	137	....	.373	.331	.704	1.019	2.430
42	139	....	.578	.290	.868	.467	2.200
43	139	....	.524	.295	.819	1.016	2.650
78	140	....	.670	.407	1.077	.679	2.833
26	143	....	.270	.282	.552	1.131	2.230
59	166	....	.782	.270	1.052	.803	2.910
61	167	....	.636	.396	1.032	.746	2.810
40	174	....	.675	.459	1.134	.541	2.810
51	175	....	.586	.511	1.097	.844	3.040
56	238	....	.464	.347	.811	.606	2.230
57	271	....	.478	.344	.822	.682	2.330
54	298	....	.869	.323	1.190	.434	2.810
Average	134	....	.531	.354	.886	.660	2.431

*Average of the four quadrants of each horn expressed in millimeters.

RESULTS

Statistical data are tabulated in Tables I, II and III. Examination of the uterine dimensions given in Table I will show that great variation was found in the diameters of normal uterine horns of the albino rat and that this variation was also manifested in the several coats of the uterine wall. It also would appear that, in the sexually mature rat, there is no direct relationship between age or body weight and these dimensions. For example, the normal horn of rat number 95, at 98 days of age and 148 grams body weight, showed a total uterine diameter of 2.57 millimeters, while rat number 96, at 133 days of age and 211 grams body weight, showed a horn measuring only 2.15 millimeters in diameter. The same type of variation was also encountered in measurements of the respective coats of the uterine wall.

Except in a few cases, the animals of the first series (Table II) were autopsied at regular intervals of 7 days from 33 to 180 days, inclusive. In this series, measurements of the atrophic horns taken at autopsy were compared to those secured by averaging the measurements of 30 normal horns. In the second series (Table III) the animals were autopsied in groups of two at intervals of 7 days, from 7 to 70, inclusive. In this series an opposite normal horn was used as an individual control for each atrophic horn.

In examining the findings of these two series of observations, one is struck by several facts illustrated in both. First, that atrophy was manifested, in a measurable degree, at a very early time. Second, that no animal in either series failed to show atrophy after the 14th day. Third, that the percentage of atrophy was not in proportion to the post-castration age of the animal under study but presented a wide range of fluctuation. Fourth, that the average per cent of atrophy was greatest in the endometrium (53.3 and 35.4, respectively), was somewhat less in the lumen (44.2 and 30.8, respectively), and was least in the myometrium (27.1 and 10.4, respectively).

Since the second series of animals was observed under better controlled conditions, further discussion of the dimensional results will be confined to this series. Comparison of the results of both series, however, will indicate an essential similarity. Figures 1 and 2 illustrate the degree of general atrophy as seen in rat number 78 after 49 post-castration days.

The Endometrium. In the two animals autopsied 7 days after castration there was no atrophy but an increase in the thickness of the endometrium. This might have been due to postoperative edema. The second group, however, autopsied 14 days after operation, showed a definite atrophy of this coat: one of 9.6 per cent and the other 39.1 per cent. After the 14th day the atrophy persisted but the degree varied greatly, reaching a maximum of 61.8 per cent on the 56th day. There was manifested a slight tendency toward increased atrophy with increased post-castration age but the increase was not regular, being marked by wide fluctuations. The average per cent for the 18 rats of the series was 35.4.

The Myometrium. Changes in the thickness of the myometrium were much less marked and, in some animals, there was no atrophy found in this coat. The maximum per cent of 32.6 was reached on the 21st day and there was seen the same type of fluctuations noted in the endometrium. The average for the 18 rats in the series was 10.4 per cent.

The Lumen. Accurate estimation of lumen changes was difficult. This was due in part to the irregularity in shape of the lumen, which varied from a fairly oval and regular outline to that of an elongated slit of varying diameter, and, in part, to the fact that even the normal lumina varied greatly in size and contour. There was noted, however, an early and definite decrease in the size of the lumina of the castrates as compared to the normals. Only rat number 92, with a post-castration age of 21 days,

TABLE II

MEASUREMENTS* OF UTERINE HORNS REMOVED AT PERIODIC INTERVALS FOLLOWING OVARIECTOMY; AVERAGES OF 30 NORMAL HORNS USED AS CONTROLS

Number	Age at Operation (days)	Age at Autopsy (days)	Postcastration (days)	Endometrium			Myometrium			Total Wall			Lumen Only			Total Uterus		
				Castrated	Control	% Atrophy	Castrated	Control	% Atrophy	Castrated	Control	% Atrophy	Castrated	Control	% Atrophy	Castrated	Control	% Atrophy
55	238	271	33	.434	.531	18.3	.331	.354	6.5	.765	.885	13.7	.606	.66	8.2	2.14	2.431	12.0
21	67	109	42	.164	.531	69.1	.191	.354	46.0	.355	.885	59.9	.303	.66	54.1	1.18	2.431	51.5
22	67	123	56	.185	.531	65.2	.224	.354	36.7	.409	.885	53.8	.295	.66	55.3	1.11	2.431	54.4
47	166	229	63	.295	.531	44.4	.237	.354	33.1	.532	.885	39.9	.418	.66	36.7	1.48	2.431	39.1
23	67	137	70	.151	.531	71.6	.237	.354	33.1	.388	.885	56.2	.352	.66	46.7	1.13	2.431	53.6
37	109	186	77	.270	.531	49.2	.208	.354	41.2	.478	.885	46.1	.418	.66	36.7	1.37	2.431	43.7
39	109	193	84	.249	.531	53.1	.278	.354	21.5	.527	.885	40.5	.303	.66	54.1	1.36	2.431	44.0
60	107	198	91	.306	.531	42.4	.360	.354	None	.666	.885	24.8	.401	.66	39.3	1.73	2.431	28.8
25	143	241	98	.196	.531	63.1	.222	.354	37.3	.418	.885	52.8	.319	.66	51.7	1.16	2.431	52.2
41	114	219	105	.295	.531	44.4	.401	.354	None	.696	.885	21.5	.180	.66	72.7	1.57	2.431	35.4
27	143	255	112	.224	.531	57.8	.213	.354	39.8	.437	.885	50.7	.319	.66	51.7	1.19	2.431	51.0
44	120	246	126	.232	.531	56.3	.270	.354	23.7	.502	.885	43.3	.442	.66	30.0	1.45	2.431	40.3
29	165	305	140	.321	.531	39.5	.211	.354	40.4	.532	.885	40.0	.311	.66	52.9	1.37	2.431	43.6
50	120	300	180	.151	.531	71.6	.286	.354	19.2	.437	.885	50.7	.467	.66	29.2	1.34	2.431	44.8
Av.	124	215	91	.248	.531	53.3	.262	.354	27.1	.510	.885	42.4	.367	.66	44.2	1.39	2.431	42.4

*Average of the four quadrants of each horn expressed in millimeters.

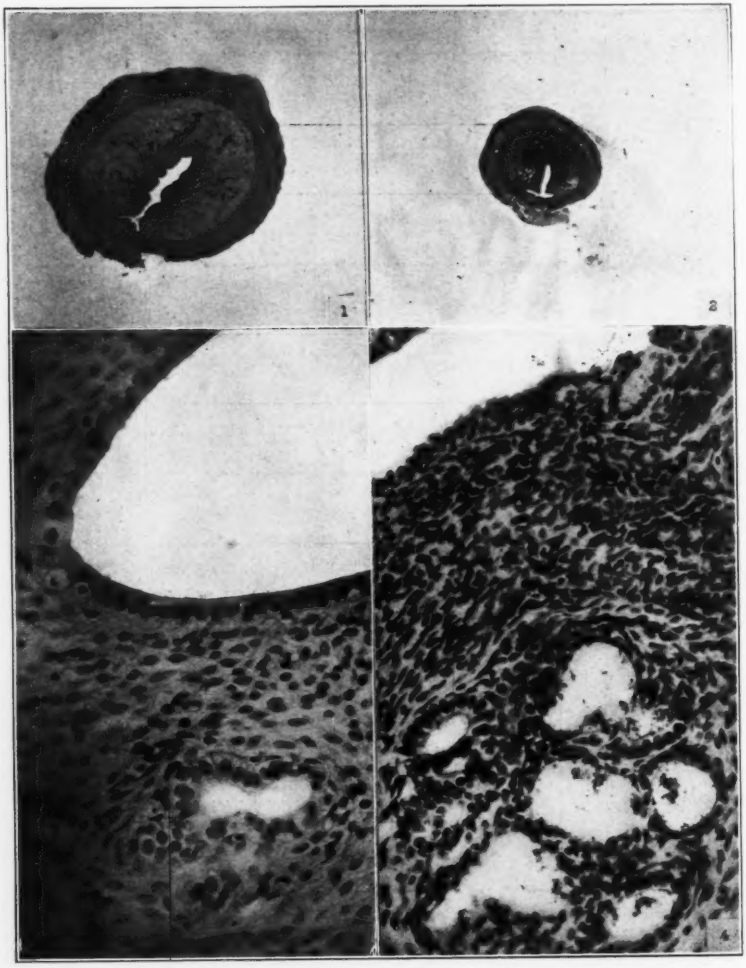
TABLE III

MEASUREMENTS* OF UTERINE HORNS REMOVED AT PERIODIC INTERVALS FOLLOWING OVARIECTOMY: OPPOSITE HORNS USED AS CONTROLS

Number	Weight at Operation (grams)	Age at Operation (days)	Age at Autopsy (days)	Postcastration (days)	Endometrium			Myometrium			Total Wall			Lumen Only			Total Uterus		
					Castrated	Control	% Atrophy	Castrated	Control	% Atrophy	Castrated	Control	% Atrophy	Castrated	Control	% Atrophy	Castrated	Control	% Atrophy
77	163	113	120	7	.505	.467	None	.421	.403	None	.928	.870	None	.507	.833	39.1	2.366	2.562	7.7
83	186	113	120	7	.433	.413	None	.389	.328	None	.823	.741	None	.546	.639	14.6	2.197	2.118	None
90	186	104	118	14	.320	.354	9.6	.339	.356	4.8	.659	.711	7.3	.452	.518	12.8	1.770	1.939	8.7
91	185	104	118	14	.370	.608	39.1	.323	.348	7.2	.693	.956	27.5	.636	.754	15.7	2.021	2.656	23.9
92	185	104	125	21	.295	.392	24.7	.262	.359	32.6	.557	.751	25.8	.508	.344	None	1.626	1.846	11.9
98	188	104	125	21	.354	.528	32.9	.319	.334	4.5	.674	.862	21.8	.362	.438	17.4	1.709	2.162	21.0
86	184	113	141	28	.408	.507	19.5	.351	.344	None	.759	.851	10.8	.470	.685	31.4	1.989	2.392	16.9
89	173	104	132	28	.326	.493	33.9	.275	.344	20.1	.602	.838	28.2	.328	.495	33.7	1.534	2.172	29.4
94	185	133	168	35	.274	.603	54.6	.261	.290	10.0	.535	.893	40.1	.528	.666	20.7	1.598	2.452	34.8
96	211	133	168	35	.385	.528	27.1	.295	.287	None	.680	.815	16.6	.282	.521	45.9	1.643	2.152	23.7
97	185	104	146	42	.223	.405	44.9	.292	.380	23.2	.515	.785	34.4	.257	.552	53.4	1.290	2.126	39.3
82	179	105	147	42	.397	.661	39.9	.392	.393	None	.789	1.054	25.1	.439	.615	28.6	2.016	2.725	26.0
78	196	140	189	49	.300	.670	55.2	.300	.407	26.3	.600	1.077	44.3	.290	.679	57.3	1.492	2.833	47.3
85	184	103	152	49	.329	.585	43.8	.353	.456	22.6	.682	1.041	34.5	.533	.836	36.2	1.897	2.918	35.0
93	171	98	154	56	.256	.408	61.8	.339	.328	None	.595	.736	19.2	.426	.574	25.8	1.616	2.046	21.0
95	148	98	154	56	.326	.608	46.4	.326	.356	8.4	.652	.954	32.4	.295	.639	53.8	1.600	2.567	37.7
79	195	103	166	63	.264	.572	53.8	.349	.367	4.9	.613	.939	34.7	.384	.611	37.2	1.610	2.490	35.3
84	197	103	173	70	.279	.557	49.9	.330	.423	21.9	.608	.980	38.0	.436	.634	31.2	1.652	2.595	36.3
Av.	183	110	145	35	.336	.519	35.4	.328	.361	10.4	.664	.880	24.5	.426	.613	30.8	1.757	2.375	25.3

*Average of the four quadrants of each horn expressed in millimeters.

failed to show some decrease in the size of the lumen. In rat number 77 the horn of the castrate showed an atrophy of 39.1 per cent 7 days after ovariectomy. The minimum of 12.8 was noted on the 14th day and the maximum of 57.3 per cent on the 49th day. Fluctuations were marked with



EXPLANATION OF FIGURES

1. Photomicrograph of section from right normal uterine horn (rat No. 78), taken at ovariectomy. (Low power).

2. Photomicrograph of section from left horn of the same rat, taken 49 days after castration. (Low power).

3. Photomicrograph of section of uterine horn (rat No. 95), showing normal histological structure. (High power).

4. Photomicrograph of section of uterine horn (rat No. 79), taken 63 days after castration. The lumen appears smaller, the epithelium flattened, the glands shruken or cystic, the cellular stroma more compact and there is some invasion of the endometrium by fibrous tissue. (High power).

slight, or no, general tendency toward a relative increase with increasing post-castration age. In fact, animals of the same pre-operative ages and of practically the same general body weight showed varying degree of atrophy (e.g., numbers 92 and 98). The average per cent of atrophy in the lumen was 30.8. The lumina were practically devoid of contents.

Total Diameter of the Uterus. The total diameter of the uterus including both walls and the lumen showed a definite, measurable atrophy after the 14th day. At no stage thereafter was there any increase in the diameters of the horns of the castrates. The minimum per cent of atrophy (8.7) was shown on the 14th day and the maximum (47.3) on the 49th day. While a general trend toward increasing atrophy with increasing post-castration age was noted, this increase was not constant. The average for the 18 animals in the series, ranging from 7 days to 70 days post-castration, was 25.3 per cent.

While the fluctuations in the degree of atrophy noted in the coats of the uterus, in the lumen, and in the total uterine horn seemed great, they were no greater than the variations encountered in the normal uterine horns (Table I).

HISTOLOGICAL CHANGES

Epithelium. The epithelium of the normal uterus of the albino rat in Stage V is of the tall columnar type, having a vertical diameter of from two to five times the transverse diameter. It is occasionally ciliated. The nuclei have from one to three nucleoli and are fairly chromatic with chromatic granules discrete. There are also basal cells present but not forming a definite continuous layer. Many of these basal cells show secretory vacuoles. Occasional mitotic figures are seen.

In the castrated animals the height of the epithelium decreased to two-thirds that of normal at 21 days post-castration. It was only one-third normal height at 28 days, grading from cuboidal to flattened epithelium, with the transverse diameter greater than the vertical. After the 28th day the epithelium varied, ranging from low columnar to flattened. In a given section the height of the epithelium varied, and even in later stages some columnar cells were seen. No ciliated cells were observed in castrated animals.

With increasing post-castration time the basal cells decreased in number, and the secretory vacuoles disappeared, being entirely absent after the 84th day. The nucleoli also decreased in number and were not seen after the 70th day. The nucleus, as a whole, lost its active appearance and became condensed, granular, pyknotic, and deeply staining. Mitotic figures disappeared after the 33rd day.

Glands. The endometrial glands seemed to have reacted differently to castration. Those more superficially placed were affected before those more deeply placed (Figs. 3 and 4). The former presented an epithelium

resembling that of the lumen with similar intracellular changes, and showed an early tendency toward cyst-formation. Definite cysts, however, appeared only after the 42nd day. Those glands more deeply placed retained their normal appearance for a longer period except for a narrowing of their lumina. On the whole, the number of glands seemed to decrease with increasing post-castration time. The glandular epithelium varied from approximately active and normal epithelium in the more deeply placed glands to an almost flat epithelium in those showing cyst formation. Intracellular changes were those of the lumen epithelium. The number of glands becoming cystic was greater with increasing post-castration time.

Endometrial Stroma. In normal endometrial stroma the cells and connective tissue are fairly equally distributed in all areas; nuclear membranes are distinct; chromatic granules are discrete and show the active appearance of normal cells; one or more nucleoli usually are seen; and occasional mitotic figures are present. In the stroma of the castrated animals there was an early manifested invasion of the endometrium by fibrous tissue. This tissue marked off two rather distinct areas: a central cellular area, and a peripheral fibrous area. It would appear that the peripheral fibrous tissue crowded the more cellular elements toward the lumen. This idea is borne out by the fact that the number of cells in a given area of the inner zone of the castrated animals showed a marked increase over the cellular elements in a similar area of the controls. In the peripheral fibrous area the reverse was true. This invasion of fibrous tissue was noted on the 14th post-castration date. On the 42nd day, in places, it had reached the lumen and occupied about two-thirds of the total area of the endometrium. On the 77th day nearly all of the endometrium was occupied by this tissue, there being only a narrow strip of the cellular zone about the lumen; at 120 days it reached the lumen in many places. This would lead one to believe that the measurable atrophy of the endometrium, cited above, was due to a general shrinking of this coat and a crowding of the tissues toward the lumen rather than that of a loss of any cellular elements.

Along with this general shrinkage, there also appeared intracellular changes similar to those noted in the epithelial cells of the lumina and of the glands. The nuclei were smaller by as much as one-half. Their shape varied greatly: at 60 days showing circular, oval, pyramidal, or irregular shapes. Nucleoli disappeared almost wholly, there being few seen after the 42nd day. No mitotic figures were observed after the 33rd post-castration date. Chromatin granules lost their discrete nature and became fused and pyknotic, appearing as heavily stained amorphous masses. The intracellular changes appeared to progress with increasing post-castration time, being marked on the 42nd day and very striking in the later stages.

The Myometrium. The myometrium seemed to be affected the least of all the coats of the uterus. There was some fibrosis of the muscle bundles, and a general crowding of the muscular elements. The muscle

cells appeared somewhat shortened and shrunken, with nuclear elongation. There was some increase in the connective tissue of the serosa, being more pronounced on the side toward the broad ligament.

Blood Vessels. The changes in the vessel walls appeared early. In animals of the 14th post-castration day, there was noted a very definite increase in the thickness of the vessel walls, and a decrease in the size of the lumina. By the 28th day, the vessel walls appeared to be three times normal thickness, and, in places, almost without lumina. This sclerosis reached its maximum at about the 30th day, and remained practically constant thereafter.

SUMMARY

A total of 32 albino rats, approximately 4 months of age and weighing from 148 to 211 grams, were castrated under aseptic technic. Two series of observations were made. In the first, consisting of 14 animals, the uterine horns of the castrates were compared to normal uterine horns taken from 30 non-castrates. In the second, consisting of 18 animals, one horn of each castrate was compared to the opposite (normal) horn removed at the time of ovariectomy.

Each castrated rat was operated upon in stage V, or interval, of the cycle. At regular intervals, from the 7th to the 180th days, inclusive, the castrated animals were autopsied and microscopic sections were made. Measurements were taken of the endometrium, myometrium, lumen, total diameter of the uterine wall and total diameter of the entire horn. Histological study was made of sections from the uteri of each castrate and of each control.

Uterine atrophy was found to have manifested itself definitely within 14 days following castration. Approximate maximum atrophy was reached on about the 49th day. There was no apparent relationship between the degree of atrophy seen and age, body weight or post-castration age. The greatest measurable degree of atrophy appeared in the endometrium, less in the lumen and least in the myometrium.

Histological changes were consistent with the decreasing thickness of the several coats. These changes consisted of a relative increase in fibrous tissue, a general shrinkage of cellular elements of the respective coats and a nuclear degeneration of these elements. General fibrosis of the vessel walls was prominent.

Uterine horns taken from non-castrated rats of similar age and body weight showed a wide variation in the thickness of the several coats.

REFERENCES

1. Kleinwachter, L.: Ztschr. f. Geburtsh. u. Gynäk. **17**: 43. 1889.
2. Eckardt: Centralbl. f. Gynäk. **20**: 786. 1896.
3. Allen, E., B. F. Francis, L. L. Robertson, C. E. Colgate, G. G. Johnston, E. A. Doisy, W. B. Kountz and H. V. Gibson: Am. J. Anat. **34**: 133. 1924.
4. Allen, W. M.: Am. J. Physiol. **92**: 612. 1930.
5. Hisaw, F. L. and S. L. Leonard: Am. J. Physiol. **92**: 574. 1930.

6. Allen, E.: Contrib. Embryol. (No. 98) **19:** 1. 1927.
7. Halban, J.: Monatschr. f. Geburtsh. u. Gynäk. **12:** 496. 1900.
8. Buys and Vandervelde: Ann. Soc. belge de chir. **1:** 393. 1893-94; Arch. de tocol. et de gynec. **21:** 372. 1893-94.
9. Carmichael, E. S. and F. H. A. Marshall: Proc. Roy. Soc. (Lond.) **76:** 387. 1907.
10. Shattock, S. G.: Proc. Roy. Soc. (Lond.) **3:** 51. 1909-10.
11. Marshall, F. H. A. and W. A. Jolly: Edinburgh M. J. **21:** 218. 1907.
12. Long, J. A. and H. M. Evans: Anat. Rec. **18:** 241. 1920.
13. Long, J. A. and H. M. Evans: Memoirs University of California, **6.** 1922.

ACTION OF THE ANTERIOR PITUITARY-LIKE SUBSTANCE OF URINE ON THE METABOLISM OF DOGS*

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DETROIT

Although preparations of the anterior pituitary-like substance of urine act chiefly upon the genital tract, there is evidence that other activities characteristic of anterior pituitary extracts are present. Loeb (1) found that the substance from pregnancy urine did not produce thyroid hypertrophy in guinea pigs. Similarly Schockaert (2) found the urine substance to be without effect on the histology or composition of the thyroid gland in ducks. Collip, Selye, Thomson, and Williamson (3) surmised that the contradictory results with respect to the action of the substance from pregnancy urine on the anterior lobe of the pituitary might be due to initial stimulation and subsequent regression analogous to the effect observed on the ovaries. They, therefore, examined treated rats at the time of the maximum effect on the ovary and found great enlargement of the anterior lobe of the pituitary, together with marked enlargement and hyperplasia of the thyroid. These effects on the pituitary and the thyroid were, however, not observed in treated male rats. Leiby (4) has also observed some increase in the size of the pituitaries and adrenals of castrated female rats treated with urinary hebin.

Since adult female dogs show extensive changes in their nitrogen, water, and energy metabolism in response to treatment with an anterior lobe preparation (5) the writer considered it of interest to study the action of the substance from urine in the same animals, to determine whether it produced any physiological responses similar to those mentioned above, either directly, or indirectly by acting upon the anterior lobe of the pituitary.

PROCEDURE

Three normal adult female dogs were used. These had already served for the previous experiments with "Antuitrin-G" above referred to, and were known to give the characteristic responses to this preparation—extensive nitrogen storage, a marked increase in metabolism associated with a fall in the basal respiratory quotient, and a considerable increase in water intake. The latter is followed by diuresis, and results in a sudden gain in weight which is lost again in the course of 8 to 10 days. The experimental procedures were the same as those in the preceding study, with the exception that a new type of metabolism mask (6) was used. The preparation injected was "Antuitrin-S," which was supplied by Parke, Davis & Co.

*Read at the Eighteenth Annual Meeting of the Association for the Study of Internal Secretions, Cleveland, June 11, 1934.

This solution was given for one day only, in a 20 cc. dose (2000 rat units), injected subcutaneously in several portions. No local reaction occurred at the site of injections.

RESULTS

Nitrogen, Water, and Energy Metabolism. In the first part of Table I the effects of a 20 cc. injection of Antuitrin-S are shown. There was a large loss of nitrogen on the day of injection. Nitrogen in the preparation approximates 1 mg. per 20 cc., and is therefore negligible. On the first day after the injection the urine nitrogen returned to its previous level. The water intake and urine volume showed no marked changes. The metabolism was determined two hours after the injection, and again one-half hour later, and was definitely elevated. The rectal temperature had, however, increased by 1.7° above that of control days, and both respiration rate and ventilation rate showed moderate, but not progressive increases. On the following day all of these effects had disappeared, while in the case of Antuitrin-G the effect on energy metabolism reached its maximum 24 to 48 hours after the injection and persisted for 6 to 8 days. The high quotients in this animal are referable to the relatively large carbohydrate intake.

In the second experiment recorded in Table I the absence of any rise in metabolism or fall in quotients during the days following the injection is again quite apparent. However, several days after the injection the

TABLE I

NITROGEN, WATER, AND ENERGY METABOLISM AFTER INJECTION OF THE ANTERIOR PITUITARY-LIKE SUBSTANCE

DOG 6

Date	Urine Nitrogen gms.	Water Intake† cc.	Urine Volume cc.	Weight kilos	Calories per Hour (basal)	Non-Protein R. Q.	Subcutaneous Injections
1932							
Dec. 27	9.15	470	460	16.4	18.9	1.00	
28	9.48	460	420	16.4	18.7	1.01	
29	11.35	485	445	16.4	23.5*	1.05	20 cc. No. 848794
30	9.93	650	590	16.3	19.5	1.00	
31	9.03	750	450	16.6	18.7	0.96	

DOG 4

1934							
Jan. 18	8.05	690	475	25.9	25.6	0.86	
19	7.95	380	390	25.7	25.4	0.84	
22	8.53	620	490	25.9	25.1	0.87	20 cc. No. 095428D
23	8.08	550	410	25.9	25.6	0.82	
24	6.75	540	550	25.9	....	....	
25	6.76	815	660	26.0	25.6	0.84	
26	7.00	850	750	26.0	25.5	0.82	
27–28	6.65	890	760	26.0	....	....	
29	5.32	720	585	26.0	....	....	
30	6.85	610	535	26.0	27.5	0.83	
31	7.49	500	535	26.1	25.3	0.91	

*Average of periods beginning 2 and 2½ hours after injection; rectal temperature, 101.7°.
†Exclusive of water present in or available from oxidation of food; see diets.

Diets: Dog 6—Carbohydrate, 110 gm.; fat, 24.3 gm.; protein, 62.7 gm., a total of 934 calories and 10.02 gm. nitrogen per day, water present or available from oxidation, 287 cc.

Dog 4—Carbohydrate, 82.6 gm.; fat, 19.6 gm.; protein, 55.3 gm., a total of 747 calories and 8.84 gm. nitrogen per day, water present or available from oxidation, 256 cc.

urine nitrogen fell markedly, and there were slight increases in water intake, urine volume, and body weight. At this time there was some enlargement of the labia, and moderate swelling about the urethral outlet and cervix, but the swelling was never so extensive as in spontaneous estrus, and instead of increasing to a maximum in a few days and progres-

TABLE II

EXPERIMENTS IN WHICH ESTRUS OCCURRED

DOG 1

Date	Urine Nitrogen‡ gms.	Water Intake‡ cc.	Urine Volume cc.	Weight kilos	Calories per Hour (basal)	Non-Protein R. Q.	Treatment and Remarks
1933–1934							
Oct. 2	8.45	680	605	27.3	28.4	0.81	
3	8.32	640	540	27.3	....	....	
4	8.31	530	580	27.2	28.3	0.82	
5	9.69	920	835	27.2	29.7	0.85	20 cc. No. 095347A
6	8.93	780	685	27.2	29.0	0.81	
7	8.60	880	810	27.2	27.9	0.79	
8	8.67	860	650	27.3	....	....	
9	8.21	1000*	870	27.3	28.2	0.75	Labial enlargement
10	7.78	1000*	875	27.4	27.9	0.76	Bleeding
11	7.55	1000*	940	27.4	....	....	
12	8.02	1000*	950	27.4	....	....	
13	8.78	1000*	1000	27.4	....	....	
14–15	9.70†	1490	1180	27.8	....	....	
16–17	8.53	1210	1140	27.7	....	....	
18–19	8.68	1225	1120	27.7	....	....	
20–22	9.07	1070	1065	27.4	....	....	
23–24	10.74	1140	1215	26.8	27.9	0.81	
25–26	10.45	770	575	27.0	....	....	Swelling subsiding
27–29	10.12	500	450	26.9	....	....	
30–31	9.61	450	400	27.0	....	....	
Nov. 1– 3	9.65	465	460	26.9	....	....	
4– 6	9.17	480	455	26.9	....	....	
7– 9	9.95	520	495	26.6	....	....	
10–12	9.33	460	400	26.6	....	....	
Dec. 18	8.33	580	450	27.0	26.2	0.93	
19	8.86	480	540	26.9	....	....	
20	8.83	....	475	26.8	....	....	
21	8.44	510	420	26.9	25.4	0.87	
22	8.46	440	395	26.8	....	....	
26	8.29	630	440	26.9	27.3	0.84	
27	8.61	750	590	27.0	28.4	0.87	20 cc. No. 095428D
28	7.98	780	725	27.0	27.8	0.77	
29	7.83	890	725	27.1	28.2	0.83	
30–31	7.63	850	725	27.2	27.5	0.78	
1934							
Jan. 1	7.53	1000*	980	27.2	....	....	
2	7.67	1250	995	27.2	29.5	0.85	
3	7.82	1160	1125	27.2	27.8	0.81	
4	8.80	970	970	27.2	27.3	0.85	
5	9.14	740	635	27.2	28.1	0.83	
6	....	....	....	27.1	....	....	Bleeding stopped
7	10.24	1020	1090	26.6	....	....	Diarrhea
8	11.45	1180	1190	26.6	....	....	Bleeding again
9	9.92	1040	620	26.8	....	....	
10	9.85	580	580	26.9	....	....	
11	9.90	730	580	26.9	25.5	0.86	Bleeding stopped
12	9.56	620	590	26.9	....	....	
13–14	8.87	610	530	26.9	....	....	Swelling disappeared
15	8.51	540	495	26.9	26.0	0.87	
16	8.40	710	740	26.8	26.0	0.79	

‡The diet was identical with that of Dog 6, Table I.
*Only 1000 cc. offered.
†Urine nitrogen, water intake, and urine volume are recorded per day, whether the period is one day or more.

sively diminishing it was quite irregular. The swelling and congestion persisted for six weeks, with occasional bleeding. During this time the nitrogen excreted in the urine fluctuated between 7.2 and 7.8 gm. per day, but eventually the control level shown in Table I was reached again. The

animal had completed a spontaneous cycle two and one-half months before the injection, so the findings observed are probably not to be regarded as irregularities produced in a spontaneous cycle by the injection.

In a third animal (Table II) there was a definite relationship between the injections and estrus cycles. The animal completed a spontaneous cycle early in June. Four months later Antuitrin-S was injected, on October 5, and within four days swelling was fully developed and bleeding soon followed. The urethra was so resistant that urine was collected in periods of several days to reduce the number of catheterizations. All of the changes were the same as those seen in spontaneous estrus and followed in orderly sequence. About two months after completion of this cycle another injection was given on December 27, and another typical cycle began two days later. This ran its course in about three weeks, and on January 26 another cycle began, without any injection, so that from June to January estrus occurred four times in eight months.

After both injections the nitrogen and water metabolism showed changes which were qualitatively similar to those which the growth preparation "Antuitrin-G" produces. During the period from October 6 to 11, the urine nitrogen fell, water intake and output increased, and a gain in weight occurred. Later on, October 23 to 31, nitrogen was lost and all of the weight increase was lost again. The same sequence was seen after the second injection. However, the changes were much less pronounced than after administration of the growth preparation, and reached their maximum on the fifth or sixth day after injections, instead of the first or second day.

The effect of the first injection on heat production was again negative. The average basal heat production for the two days preceding the injection, and for the preceding week not shown in the table, was 28 calories per hour, and the five observations following the injection averaged 28.5 calories per hour. In the second experiment there was a definite effect. The six control days, Dec. 18, 21, 26, and Jan. 11, 15, and 16, averaged 26.1 calories per hour, while the eight observations following the injection averaged 28.1 calories. This result, which was the only positive one out of four, was therefore dependent on lower basal values, since the heat production after the injection was no greater than in the preceding experiment. A number of low quotients were noted during both of the periods after injections.

The lower basal values in the second experiment appear to be due to a seasonal variation and not to the experiments. Two months later, in March, 1934, the basal values had fallen further to an average of 24.8 calories per hour, which, allowing for surface area, corresponded to the values during the two preceding years. The basal values during the Spring months of 1932, 1933, and 1934 were 663, 675, and 683 calories per square meter per day. The control levels in the above experiments calculate to 769 and 720 calories per square meter per day. The diet was unchanged throughout

the three-year period, and the room temperature at which all determinations were made was always within a degree of 28° C. The surface area was calculated from weight and length according to Cowgill and Drabkin (7).

Estrus. The regular occurrence of estrus in Dog 1 (Table II) after administration of the substance from pregnancy urine was of some interest, and further experiments were carried out without metabolism studies. With one exception, all of the results are summarized in Table III. The

TABLE III

ESTROGENIC EFFECTS OF A.P.L. IN DOGS

Dog No.	Date of Injection	Dose	Time Since Last Estrus	Result
1	10- 5-33	20 cc.	4 months	Estrus beginning in 4 days.
1	12-27-33	20 cc.	2 months	Estrus beginning in 2 days.
4	1-22-34	20 cc.	2½ months	Enlargement of labia, swelling about urethral and cervical outlets, irregular bleeding, duration of swelling, 2½ months.
8	3-10-34	20 cc.	1½ months	Enlargement and hyperemia of labia, some swelling about urethral outlet, duration, 2 weeks.
8	5-19-34	20 cc.	3½ months	Same effects repeated.
9	1-25-34	20 cc.	.....	Transient swelling, slight bleeding, 3 days.
9	2-20-34	20 cc.	.....	No effect.
9	3-10-34	20 cc.	.....	No effect.
10	2-20-34	20 cc.	.....	No effect.
10	3-10-34	20 cc.	.....	Transient swelling.
10	4- 5-34	20 cc.*	.....	No effect.
10	5-19-34	20 cc.	.....	No effect.

*Given in doses of 1 to 2 cc. daily.

experiment on Dog 6, Table I, is omitted, since estrus was in progress at the time. The time of the last spontaneous cycle preceding the injection was known in three of the five animals, and all five were non-pregnant. Typical estrus cycles beginning two to four days after the injections were noted only in Dog 1. Swelling and hyperemia of the labia was commonly observed, with or without varying degrees of swelling and congestion about the urethral and cervical outlets.

DISCUSSION

From the preceding experiments it is clear that the anterior pituitary-like substance of urine, administered in large single doses, does not have a "thyrotropic" action such as is observed when alkaline extracts of beef anterior lobes are administered in the same way. In four experiments only one showed a sustained increase in metabolism. The one positive result could not be ascribed to occurrence of estrus, since estrus induced with oestrin has no effect on the metabolism of dogs (8). It is possible that more positive results would be obtained if the experiments were carried out when the basal values reach their lowest point, but the anterior pituitary preparation produced its calorigenic action at all times.

Similarly, the immediate and drastic effects on the water and nitrogen metabolism observed in the case of alkaline extracts of beef anterior lobes

were not produced by the anterior pituitary-like substance of urine. The moderate and delayed effects of this type which occurred in several experiments suggest the possibility of an indirect action by way of the pituitary, but this cannot be definitely decided without hypophysectomy.

SUMMARY

1. Single doses of approximately 2000 rat units of anterior pituitary-like substance from pregnancy urine had no prolonged effect on the basal metabolism of three dogs.

2. Changes simulating the action of growth preparations of beef anterior lobes—water and nitrogen storage, with subsequent loss of both—occurred in 3 experiments, but were delayed and less marked than those which followed administration of growth hormone preparations.

3. Enlargement of the labia and other signs of estrus were commonly seen after injections of the above dosage. Induction of estrus was observed in only one of the five dogs.

REFERENCES

1. Loeb, L.: The specificity in the action of the anterior pituitary of different mammals as well as of urine of pregnant women on the sex organs and thyroid glands of immature female guinea pigs. Endocrinology, **16**: 129. 1932.
2. Schockaert, J. A.: Hyperplasia of thyroid and exophthalmos from treatment with anterior pituitary in young duck. Proc. Soc. Exper. Biol. & Med. **29**: 306. 1931.
3. Collip, J. B., H. Selye, D. L. Thomson and J. E. Williamson: Effect of prolonged administration of the anterior pituitary-like hormone on pituitary and thyroid. Proc. Soc. Exper. Biol. & Med. **30**: 590. 1933.
4. Leiby, G. M.: Effect of antuitrin-S on weights of the pituitary, adrenal and thyroid. Proc. Soc. Exper. Biol. & Med. **31**: 14. 1933.
5. Gaebler, O. H.: Some effects of anterior pituitary extracts on nitrogen metabolism, water balance, and energy metabolism. J. Exper. Med. **57**: 349. 1933.
6. Gaebler, O. H.: Metabolism masks for dogs. Proc. Soc. Exper. Biol. & Med. **31**: 500. 1934.
7. Cowgill, G. R. and D. L. Drabkin: Determination of a formula for surface area of the dog together with a consideration of formulae available for other species. Am. J. Physiol. **81**: 36. 1927.
8. Kunde, M. M., F. E. D'Amour, A. J. Carlson and R. G. Gustavson: The effect of estrin injections on the basal metabolism, uterine endometrium, lactation, mating, and maternal instincts in the adult dog. Am. J. Physiol. **95**: 630. 1930.

CLINICAL OBSERVATIONS ON THE EFFECTS OF APL (ANTUITRIN-S) ON THE TESTICLE*

W. L. BROSIUS, M.D.
DETROIT

The development of clinical endocrinology and the ultimate utilization of the vast amount of information gathered by the experimentalists and biochemists depends on the accumulation of supplementing and complimenting observations on the human species.

Man's desire to postpone senility and prolong the reproductive period has been one of the chief incentives to the investigation of the glands of internal secretion.

Castration of animals and human beings has been practiced and observed since earliest times, but the record of its results in man contains so many conflicting statements and is so clouded with superstition and perpetuated misinformation that standards for critical judgment are lacking. Consequently, the valuation of the results of replacement therapy and attempted stimulation has been hindered.

Spermatogenesis and testicular descent are objective criteria which can be observed and recorded with sufficient accuracy to form a basis for measuring the effects of stimulation of the pituitary-gonad portion of the reproductive mechanism.

Stimulation of the gonads in animals has been accomplished with material from two sources: first, the anterior lobe of the pituitary gland by implantation as well as by the administration of the gland substance and extracts prepared from it; second, by the anterior-pituitary-like substances from urine which, for convenience, have been called A.P.L. One of these is found in the urine of pregnancy of the human and of a few other animals and has been called P.U. Another factor found in the urine after castration and the menopause has been called F.S.U. (1) because of the active stimulation of follicle formation which it causes.

Numerous preparations from gland and urine sources have appeared on the market attractively advertised to the medical profession by mail, by detail, and by sample. The exploitation of therapeutic agents of questionable potency and dubious merit, and their consequent wide-spread use by non-critical physicians in uncertain dosages and more uncertain diagnoses can only yield a high percentage of disappointing results.

Agents of assayed potency should be used according to our current knowledge of indications and dosage with as much control as it is possible to exercise in clinical medicine, and the results obtained, bad, negative, and good, should be faithfully reported.

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The therapeutic agent used in the study reported here was Antuitrin-S containing 100 rat units of P.U. per cc. It was injected into the gluteal muscle.

ASPERMIA

As previously reported (2), in a case of complete aspermia with bilateral testicular atrophy following orchitis as a complication of mumps, motile spermatozoa appeared after the administration of P.U. (Antuitrin-S). Increases and decreases in the number of spermatozoa and in their motility have for three years repeatedly paralleled increases and decreases in the intensity of treatment.

From August, 1933, to February, 1934, the patient received 2 cc. (200 units) once a week. A condom specimen examined December 18, 1933, showed a few spermatozoa, about 25 per cent of which were motile. On February 2 an attack of appendicitis necessitated the removal of a subacute retrocaecal appendix. No. P. U. was administered for the next two months.

On March 24, 1934, a condom specimen was examined 90 minutes after it was obtained. Its amount was 5 cc. and it contained 350 spermatozoa per cu. mm. None were motile. A condom specimen was examined on March 31, 75 minutes after it was obtained. Its amount was 5 cc. and it contained 330 spermatozoa per cu. mm. None were motile. The right testis measured 38 by 25 mm. and the left measured 37 by 23 mm. The prostate was normal in size and consistency. Administration of Antuitrin-S 2 cc. twice weekly was begun.

On May 1, 1934, after the patient had received a total dosage of 20 cc., a condom specimen was examined 75 minutes after it was obtained. Its amount was 5 cc. and it contained 682 spermatozoa per cu. mm. Motility was observed in about 1 per cent.

On May 29, 1934, after a total dosage of 34 cc. had been given, a condom specimen was examined 90 minutes after it was obtained. Its amount was 5 cc. and it contained 2,568 spermatozoa per cu. mm. About 3 per cent were motile and maintained their motility in a cover slip preparation at room temperature for one hour but were non-motile at the end of the second hour. The right testis measured 40 by 30 mm. and the left testis measured 39 by 30 mm. The consistency was slightly firmer than at the previous examinations. The prostate was unchanged in size and consistency.

Microscopic examination of fresh and stained preparations from the four specimens showed from 80 per cent to 85 per cent with normal morphology (3). Occasional forms were seen in which two head pieces had not separated. These were interpreted as evidence of incomplete maturation (4).

The report of this case has made possible the collection of data on 12 cases of male sterility treated by seven physicians, to whom I am indebted for the material for the following report.

Case No. 1. Age 31 years. No spermatozoa were present in condom specimens or vesicle strippings. The cause and duration of the condition were unknown. There was no testicular atrophy. Libido and potentia were normal. The prostate was normal in size and consistency. Material massaged from the prostate contained an occasional polymorphonuclear leucocyte.

The patient received 1 cc. of Antuitrin-S approximately twice weekly for 21 weeks. The total amount administered was 33 cc. Condom specimens examined 6, 12, and 23 weeks after therapy was begun were reported as containing, respectively, numerous, few, and many non-motile spermatozoa.

Case No. 2. Age 45 years. The patient has had complete absence of erection and ejaculation since an attack of mumps complicated by a left orchitis with resultant atrophy six years ago. Libido remained normal. The prostate was normal in size and consistency. In addition he complained of frequent severe headaches, extreme nervousness and marked weakness.

He received 1 cc. of Antuitrin-S twice weekly for three weeks, then 2 cc. twice weekly for six weeks, a total of 30 cc. His atrophied testis increased to almost the size of the normal one and he was able to maintain a fair erection for a short time but had no ejaculation. He was entirely free from headaches, nervousness and weakness.

Case No. 3. Age 30 years. The patient had been impotent since an attack of mumps complicated by bilateral orchitis with resultant bilateral testicular atrophy fifteen years before. His prostate was atrophic and he had an inferiority complex.

He received 2 cc. of Antuitrin-S twice weekly for eight weeks, a total of 32 cc. There was an increase in size of the testes from 2 cm. to 2½ cm. Otherwise the results of the treatment were negative.

Case No. 4. Age 39 years. There had been complete aspermia for twenty years. The cause was reported as probably mumps. Both testes were atrophic. Libido and potentia were slightly subnormal.

He received 2 cc. of Antuitrin-S three times weekly for five weeks, a total of 30 cc. No results of any kind were observed.

Case No. 5. Age 35 years. Condom specimens showed no spermatozoa. The cause and duration of the condition were unknown. Both testes were small. Libido, potentia, and the prostate were normal.

He received 1 cc. of Antuitrin-S four times a week for six weeks. The total amount administered was 23 cc. There was no change in the specimen examined at the end of the treatment.

Case No. 6. Age 35 years. Condom specimens and vesicle strippings contained many non-motile but no motile spermatozoa. The cause and duration of the condition were unknown. There was no testicular atrophy. Libido and potentia were normal. The prostate was normal in size and consistency. Material massaged from the prostate contained an occasional polymorphonuclear leucocyte.

He received 1 cc. of Antuitrin-S approximately twice weekly for twelve weeks. The total amount administered was 18 cc. A condom specimen examined at the end of treatment was reported as containing many non-motile and a few slightly motile spermatozoa.

Case No. 7. Age 27 years. He had been married two and one-half years, had used no contraceptive measures, and there had been no pregnancy. Condom specimens and vesicle strippings contained a few non-motile but no motile spermatozoa. The cause and duration of the condition were unknown. There was no testicular atrophy. Libido and potentia were normal. The prostate was normal in size and consistency. Material massaged from the prostate contained no polymorphonuclear leucocytes.

He received 1 cc. of Antuitrin-S twice weekly for six weeks, a total of 12 cc. Treatment was stopped at this time because his wife was pregnant. The pregnancy is progressing normally at the fourth month.

Case No. 8. Age 32 years. Condom specimens contained many non-motile but no motile spermatozoa. The cause and duration of the condition were unknown. There was no testicular atrophy. Libido and potentia were normal. The prostate was reported as congested.

He received 1 cc. of Antuitrin-S twice weekly for five weeks, a total of 10 cc. A condom specimen examination at the end of treatment showed many active spermatozoa. His physician states, "This patient had been a heavy drinker but in the past month has been 'on the wagon' so this may have had some influence on the improvement of his specimen."

Case No. 9. Age 32 years. The condom specimen contained a very few non-motile but no motile spermatozoa. The condition followed influenza complicated by bilateral orchitis with resultant bilateral testicular atrophy fifteen years ago. Libido and potentia were normal. The prostate was normal in size and consistency.

He received 1 cc. of Antuitrin-S twice weekly for five weeks, a total of 10 cc. Condom specimens examined at the third and fifth weeks showed no increase in number of spermatozoa and no motility. The only change noticed was a slight tenderness in the testes that was not present before.

Case No. 10. Age 35 years. The condom specimen contained occasional non-motile but no motile spermatozoa. No history of the cause of the condition could be elicited.

He received 2 cc. of Antuitrin-S twice weekly for seven weeks (the initial dose was 1 cc.), a total of 27 cc. The report of his condition at the end of treatment was, "No sign of improvement."

Case No. 11. Age 31 years. Repeated examinations of condom specimens showed a moderate number of spermatozoa. They were usually non-motile although a few were observed showing very sluggish motility. The cause and duration of the condition were unknown. There was no testicular atrophy. Libido and potentia were normal. The prostate was normal in size and consistency.

He received 2 cc. of Antuitrin-S three times weekly for 19 weeks, a total of 114 cc. After 12 weeks of treatment his condition was absolutely unchanged. After 19 weeks of treatment there was increase in motility, and motility was observed on the slide as long as two hours after ejaculation. His physician closes his report with the statement, "The condition is enormously improved from the time he began treatment."

Case No. 12. Age 30 years. He was reported to have active spermatozoa and small, soft testes. His libido was described as under par and he was said to be impotent. His prostate was small.

He received 1 cc. of Antuitrin-S three times weekly for four weeks, then 1 cc. daily for two weeks, a total of 26 cc. Since treatment he has had satisfactory intercourse several times. The testes are apparently increased in size and are more firm. His physician states, "This patient was a neurasthenic to begin with so I feel that I must be guarded in the amount of credit that I give to the drug, and the amount to the psychological effect."

CRYPTORCHIDISM

The usual management of cryptorchidism has been surgical. Schapiro (5) treated 13 subjects with this condition with P.U. and reported improvement in all. Engle (6) hastened the descent of testes in immature monkeys by P.U. administration. Sexton (7) reported treatment with P.U. of six boys with cryptorchidism. In four cases there was complete descent.

On the basis of these reports the treatment of the nine cases here reported was undertaken. For the data in five of the cases I am indebted to four physicians for permission to use their records.

Case No. 1. Age 15 years 3 months. The right testis was low in the inguinal canal, the left testis in normal position. The right testis was approximately the same size and consistency as the left. The patient was 66.4 inches tall and weighed 120 pounds. His basal metabolic rate was plus 7 per cent.

He received 3 cc. of Antuitrin-S three times weekly for three and one-half weeks, a total of 30 cc. Examination at this time showed the right testis descended through the external ring and down to the level of the pubic bone. Treatment is being continued.

Case No. 2. Age 12 years 6 months. Both testes were held tighly against the lower portion of the pubic bone and could not be forced into the scrotum. They measured 13 by 9 mm. and were soft. The penis was small and there was slight mental retardation. The patient was 57.7 inches tall and weighed 93.7 pounds. His basal metabolic rate was minus 11 per cent.

He received 2 cc. of Antuitrin-S three times weekly for 14 weeks, a total of 42 cc. He also received desiccated thyroid gland to tolerance. Examination at the end of treatment showed both testes in normal position, increased in size, measuring 16 mm. by 11 mm. and more firm.

Case No. 3. Age 11 years. The right testis was just outside the inguinal canal, over the external ring. The left testis was in the normal position.

He received 1.5 cc. of Antuitrin-S daily for 10 days, then 3 cc. daily for 15 days, a total of 60 cc. in 25 days. Examination after treatment showed no change in the position, size, or consistency of the testis.

Case No. 4. Age 10 years. The right testis was low in the inguinal canal, measured 17 by 12 mm., and was smaller and softer than the left testis which was in normal position.

He received 1.5 cc. of Antuitrin-S once weekly for five weeks, a total of 7.5 cc. Examination at the end of treatment showed the right testis in normal position and slightly increased in size and consistency.

Case No. 5. Age 9 years. Neither testis could be palpated by three examiners. The boy showed a typical neuro-hypophyseal obesity. He was 58 inches tall and weighed 111 pounds. His basal metabolic rate was minus 7 per cent.

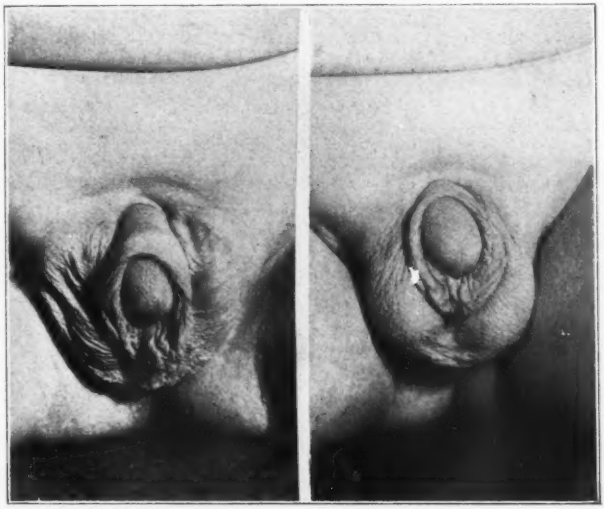

Figure 1. Case No. 5. Age 9 years. Left figure before treatment: neither testis palpable. Right figure after eight weeks' treatment with Antuitrin-S twice weekly and desiccated thyroid gland twice daily shows the testes in normal position.

He received 2 cc. of Antuitrin-S twice weekly for eight weeks, a total of 32 cc. He also received desiccated thyroid gland, grains ½, twice daily. Examination at the end of treatment showed both testes in normal position, measuring 18 by 13 mm., and of normal consistency.

Case No. 6. Age 8 years 6 months. The left testis was low in the inguinal canal and measured 14 by 10 mm. and was softer than the right testis which was normal in position and measured 15 by 12 mm. He was 49 inches tall and weighed 50 pounds. His basal metabolic rate was minus 37 per cent and minus 32 per cent.

He received 2 cc. of Antuitrin-S twice weekly for three weeks, a total of 9 cc. He also received desiccated thyroid gland, grains ½, twice daily. Examination after three injections showed the left testis below the external inguinal ring over the pubic bone. It could not be forced into the scrotum. Examination at the end of treatment showed both testes in normal position, both measuring 16 by 13 mm. and of equal consistency.

Case No. 7. Age 7 years. This boy was brought to a clinic with his brother, Case No. 8, because of a dry scaling skin similar to that of his brother, who was

improving under thyroid therapy. By error he received two injections of 1 cc. each of Antuitrin-S. He was examined eight weeks later when the left testis was just below the external inguinal ring over the pubic bone. It could be forced to just below the pubic bone but was immediately retracted to its former level. It measured 16 by 10 mm. and was softer than the right testis which was normal in position and measured 17 by 12 mm.

He received 1 cc. of Antuitrin-S on the day of examination and 14 and 17 days later, a total of 5 cc. in 10 weeks. He also received desiccated thyroid gland, grains ½, twice daily. Seven days after the last injection examination showed both testes in normal position, both measuring 17 by 12 mm. and of equal consistency.

Case No. 8. Age 4 years. The left testis was in the midportion of the inguinal canal, measured 13 by 9 mm., and was softer than the right testis which was normal in position and measured 14 by 10 mm. He had patches of dry scaling skin resembling ichthiosis, scanty hair, a speech difficulty, and was slightly obese.

He had received desiccated thyroid gland, grains ½, twice daily for three months before his undescended testis was noted. This thyroid therapy was continued during and after his treatment with Antuitrin-S.

He received ½ cc. of Antuitrin-S on the day of examination and 1 cc. seven and 11 days later. Examination 24 days after the last injection, and four, eight, and 12 weeks later, showed both testes in normal position and of equal size and consistency.

Case No. 9. Age 3 years. Neither testis could be palpated by three examiners. He received 1 cc. of Antuitrin-S on the day of examination and two days later. Three days after the second injection two examiners palpated one testis in the midportion of the inguinal canal and the other low in the canal just within the external ring. On the sixth, twentieth, thirty-fourth, and forty-eighth days after examination he received 1 cc. of Antuitrin-S, a total of 7 cc. Examination by two examiners at the time of the last injection showed one testis low in the inguinal canal just within the external ring and the other below the external ring over the pubic bone. Treatment is to be continued.

Case No. 10. Age 38 years. A hypopituitary dwarf, 61 inches tall, weighing 90 pounds, came under observation for exophthalmic goiter for which a thyroidectomy was performed. He was found to have a left testis just below the inguinal canal over the external ring. The right testis could not be palpated. It was decided to treat him and observe the left testis for enlargement or tenderness. He received nine injections of 1 cc. and 33 injections of 2 cc., a total of 75 cc. of Antuitrin-S, about five times weekly during nine weeks. No effect on the testis could be observed. There was no descent.

The small amount of treatment which some of these cases received makes it necessary to consider the possibility of spontaneous descent as described by Drake (8). The number of cases makes it improbable that the descent following treatment was coincidence.

SUMMARY

A previously reported case of aspermia in which motile spermatozoa appeared after the administration of extract of pregnancy urine has been observed for three years. During this time increases and decreases in the number of spermatozoa and their motility have repeatedly paralleled increases and decreases in the intensity of treatment. No treatment was given for two months. At the end of this period the examinations of two specimens of semen showed in one 350 and in the other 330 non-motile spermatozoa per cu. mm. Therapy consisted of 2 cc. of P.U. twice weekly. After four weeks of therapy the semen contained 682 spermatozoa per cu. mm. One per cent were motile. After eight weeks of therapy the semen contained 2,568 spermatozoa per cu. mm. Three per cent were motile.

In five cases of aspermia the subject received from 1 to 2 cc. of P.U. two to four times weekly for periods varying from five to 21 weeks. In one case non-motile spermatozoa appeared. In two cases there was increase in testis size, and one of the two subjects who had been impotent developed potentia while under treatment. In two cases the treatment was without demonstrable effect.

Five subjects with non-motile, but no motile, spermatozoa received from 1 to 2 cc. of P.U. twice weekly for periods of from five to twelve weeks. In two of these cases motile spermatozoa were demonstrated following treatment. One patient discontinued treatment when his wife became pregnant. In one case slight testicular tenderness developed. In one case the treatment was without demonstrable effect. A patient who had a moderate number of spermatozoa, a few of which showed sluggish motility, was given 2 cc. of P.U. three times weekly for 19 weeks. After treatment he had many actively motile spermatozoa.

A subject with bilateral testicular atrophy with a few motile spermatozoa and who was impotent received 1 cc. of P.U. five times weekly for four weeks, then 1 cc. daily for two weeks. During his treatment there was a progressive increase in the size and firmness of his testes and he became potent.

In nine cases of undescended testis in boys ranging in age from 15 years to three years P.U. was given in dosages varying from 1 to 3 cc. at intervals of from one to three times weekly. In one case daily injections of 1.5 cc. were given for 10 days, followed by 3 cc. daily for 15 days.

In five of the cases desiccated thyroid gland, ½ grain, twice daily was given, and in one thyroid to tolerance. One subject had been given thyroid for three months before his undescended testis was observed.

Following the treatment there was complete descent in six cases. In two cases partial descent has been attained and treatment is being continued. In one case the treatment was without demonstrable effect. A hypopituitary dwarf, 38 years old, had one testis above the pubic bone. The other testis could not be palpated. He received 1 cc. of P.U. five times weekly for nine weeks, then 2 cc. five times weekly for nine weeks, a total of 75 cc., without demonstrable effect.

CONCLUSION

The cases here reported indicate that the P.U. factor from the human urine of pregnancy stimulates spermogenesis and hastens testicular descent in the human.

For the reports of the cases of absolute and relative aspermia I am obligated to the following physicians: Cases 1, 6, and 7; Dr. F. H. Cole. Case 2; Dr. Philip P. Bonifer. Case 3; Dr. L. B. Amick. Case 4; Dr. L. M. Spaulding. Case 5; Dr. Harold B. Davidson. Cases 8 and 12; Dr. Wm. R. Delzell. Case 9; Dr. Clement L. Poston. Case 10; Dr. Stanley T. Lowe. Case 11; Dr. Terry M. Townsend.

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REFERENCES

1. Smith, P. E., E. T. Engle and H. H. Tyndale: Proc. Soc. Exper. Biol. & Med. **31:** 6. 1934.
2. Brosius, W. L. and R. L. Schaefer: J. A. M. A. **101:** 1227. 1933.
3. Moench, G. L.: Am. J. Obst. & Gynec. **25:** 410. 1933.
4. Cary, W. H. and R. S. Hotchkiss: J. A. M. A. **102:** 8. 1934.
5. Schapiro, B.: Deutsche med. Wchnschr. **56:** 1605. 1930; Ztschr. f. klin. Med. **114:** 610. 1930.
6. Engle, E. T.: Endocrinology, **16:** 5. 1932.
7. Sexton, D. L.: Endocrinology, **18:** 1. 1934.
8. Drake, C. B.: J. A. M. A. **102:** 10. 1934.

THE PROLONGED ADMINISTRATION OF THEELIN AND THEELOL TO MALE AND FEMALE RATS AND ITS BEARING ON REPRODUCTION*

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INTRODUCTION

The results obtained by subjecting male and female animals to the influence of the female sex hormone have been reported from a number of laboratories. In the case of the male, Herrmann and Stein (1916) injected young rats and rabbits with ovarian preparations and found that puberty was retarded; using larger doses degenerative changes were produced in the testes. Similar results have been obtained by Fellner (1921), Gould and Doisy (1924), Steinach and Kun (1926), Laqueur and deJongh (1928), Laqueur (1929) and others. More recent experiments by Wade and Doisy (1931), Spencer, Gustavson and D'Amour (1931, 1932), Moore and Price (1932) and others have shown that the injection of the female sex hormone caused inferior body growth and genital development. In adult male mice Allanson (1931) found that the injection of crystalline estrin for two or three weeks did not interfere with normal spermatogenesis and did not destroy fertility. On the other hand, Reiprich (1931) and others reported partial sterility in the male following the administration of estrogenic material.

In the female the administration of estrus-producing extracts has been found to replace the estrogenic functions of the normal ovary. In addition to this, other effects in which we are more interested at present, have been reported. Allen and Doisy (1924) reported inhibition in the development of the ovary of the young rat after treatment with the ovarian hormone. Later and more extensive investigation by Doisy, Curtis and Collier (1931) showed that the injection of young rats with 3 rat units of theelin daily for 5 weeks almost completely retarded ovarian development. In a few cases follicles matured normally and ovulation occurred. Animals given a single massive dose of 20 units showed recovery from the effects but ovarian development was consistently slower than in the controls by about 10 days. Similar results were obtained by Pincus and Werthessen (1933) and Bialet-Laprida (1933a). With mature animals the latter (1933b) found that the ovaries regressed towards the infantile condition following injections extending over 35 days. However, recovery was observed after cessation of injection. In dogs Kunde, et al. (1931) found a similar diminution in the size of the ovaries with an arrest of follicular development. Comparable results were also obtained by Spencer, D'Amour and Gustavson (1932), who found inferior growth and gonad development of young estrin-treated rats; fertility, however, returned after the injections were discontinued. In addition to the effects on the ovary, Leonard, Meyer and Hisaw (1931) observed that the pituitaries of young rats similarly treated contained less gonad-stimulating material. Loeb and Kountz (1928) reported that the administration of follicular extracts to young guinea pigs hastened follicular maturation but prevented ovulation. They believed that sterility should persist as long as the injections are continued. They also observed that such treatment inhibited the decidual reaction: this portion of the work being confirmed by Brouha (1932). Castillo (1932) was able to inhibit ovulation after coitus in the rabbit by the injection of folliculin and thus produce sterility. Haberlandt (1924-30) has also produced sterility in rabbits, mice and guinea pigs with similar agents and Hauptstein (1931) has reported similar results in mice.

While studying the effects on pregnancy, Parkes and Bellerby (1926) injected estrin into mice during the early stages and observed the reappearance of estrus with the termination of gestation; during late stages of pregnancy larger amounts were required. Similar results were reported by Smith (1926). Gostimirovic (1930) and D'Amour, D'Amour and Gustavson (1933). Kelly (1931)

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found that the administration of estrin to guinea pigs at any stage of gestation caused the termination of pregnancy. In late pregnancy, death of the mother invariable followed.

Estrus changes and cornification without the termination of pregnancy were produced in pregnant rats by the subcutaneous administration of estrogenic extracts (Allen, Francis and Craig, 1925). In later stages of pregnancy and during lactation, larger doses were required to elicit the reaction. Parkes and Bellerby (1927), who obtained similar results in mice, found that larger doses were required if the mothers were nursing large litters.

Experiments of a somewhat different nature were reported by Courrier (1924), who induced estrus changes in the vaginae and uteri of new-born guinea pigs by injecting the pregnant female with the estrogenic hormone. In a later publication (1932) he reported similar results in the cat. Allen, who did not observe these effects in the rat, thinks that this variation may be due to a species difference.

Brouha and Simonnet (1925) administered the estrogenic hormone to rats during late pregnancy or lactation without affecting delivery, milk secretion or genital development of the young. Somewhat later, Levin, Katzman and Doisy (1931) injected up to 500 rat units of theelol or 600 rat units of theelin during the second half of pregnancy in rats and likewise found no detectable effect on gestation, delivery or survival of the offspring. In a later publication, Katzman (1932) reported the injection of adult female rats with theelol and theelin for 25 days. A month after the cessation of injection the females mated and reproduced normally.

These reports and others based on relatively short periods of treatment leave uncertain the ultimate effect of long-continued administration of estrogenic substances on the reproductive behavior of the male and female animal. The experiments reported in this paper were conducted over an extended period to see if male and female rats could develop normal reproductive functions while under the influence of the female sex hormones, theelin and theelol.

MATERIALS AND METHODS

Theelin and Theelol. The solutions of theelin and theelol were made by dissolving the pure crystalline preparations in 95 per cent alcohol and then diluting with water to the proper concentration. A small amount of alkali was frequently added to keep the material in solution. Control injections were made with distilled water containing the same concentration of alcohol. In view of the confusion due to the great number of "biological units" in use, the values in this paper are expressed in metric units of the pure crystalline compounds.

Animals. Young male albino rats of the Wistar strain, 21-25 days of age were selected and divided into groups using litter-mates for controls. Daily subcutaneous injections of 0.65 to 32.5γ of theelol and 1.65 to 6.6γ of theelin were continued for 113 to 242 days. Body weights were recorded at 5-day intervals. After reaching sexual maturity all of the animals were mated to normal females. Usually the injected male and a control were killed after the former had impregnated his consort. After discarding a number of animals because of inner ear infection 92 animals remained from which data were secured.

Forty-one female rats 23 to 27 days of age were injected subcutaneously with from 0.65 to 32.5γ of theelol daily for 143 to 316 days. A group of 16 animals served as controls in addition to observations on the stock colony.

The general plan was to inject the animals as a group until they reached sexual maturity; at this time they were separated and mated. The injections were continued throughout the period of pregnancy and discontinued at parturition; in the event that the litter died the female was again injected as before. Smears were made daily to determine when the animals mated and to watch for indications of pregnany or pseudopregnancy. The placental sign was usually taken as evidence of pregnancy.

To observe the effect of theelol on the vaginal smears, uncomplicated by pregnancy, a group of 18 females 3 to 4 months of age were injected with from 0.65 to 13.0γ for periods up to 130 days, after a preliminary period of observation.

The effect of theelol on the spayed female was observed on a group of 23 animals about 6 months of age. Injections of 0.65 to 13.0γ were continued over a period of 180 days.

EXPERIMENTS AND RESULTS

The data bearing on the reproductive behavior of the male rats are summarized in Table I. Following the mating of these animals, 24 of the

TABLE I

ADMINISTRATION OF THEELOL AND THEELIN TO MALE RATS

Type of Animals	No. of Rats	Average Days Injected	Average Gain in Body Wt. in Gms.	Relative % Gain in Body Wt.	Average Wt. of Testes in Gms.	Average Wt. of Prostate in Gms.	Average Wt. of Cowpers in Gms.	No. Animals Producing Litters
0.65 γ theelol	11	161	208.0	86.7	2.586	0.252	0.045	7
Control	6	158	240.0	100.	2.608	0.302	0.060	
3.25 γ theelol	7	207	216.0	93.5	2.409	0.203	0.046	2
Control	7	195	231.0	100.	2.516	0.322	0.064	
9.75 γ theelol	6	188	227.0	91.9	2.424	0.275	0.050	2
Control	3	183	247.0	100.	2.424	0.261	0.059	
13.0 γ theelol	10	205	227.0	89.7	2.370	0.265	0.045	4
Control	7	184	253.0	100.	2.571	0.302	0.061	
32.5 γ theelol	5	208	202.0	84.9	2.321	0.212	0.044	1
Control	3	207*	238.0	100.	2.726	0.288	0.065	
1.65 γ theelin	5	149	198.0	86.1	2.047	0.174	0.036	2
Control	6	157	230.0	100.	2.559	0.298	0.063	
6.6 γ theelin	9	148	176.0	74.3	1.638	0.135	0.031	6
Control	7	147	237.0	100.	2.342†	0.295	0.060	
Total Experiment	53	181	208.0	87.0	2.258	0.209	0.042	24
Total Control	39	176	239.0	100.	2.535	0.295	0.062	17

* 2 rats. † 5 rats.

53 injected males produced litters compared to 17 out of 39 for the controls. Ten of the 34 experimental males which mated failed to produce litters in contrast to one for the controls. At least three of the injected animals which had infertile matings impregnated the female at a subsequent mating. It is important to observe that fertile matings were recorded from all of the groups of injected animals; and further, that 6 of the 9 animals injected daily with 6.6γ of theelin produced litters. This is of greater importance when one considers that this is the group that showed the greatest retardation in genital development and body growth.

The data given above indicate that testis function, for the most part, was normal. At autopsy the reproductive organs were removed and the various weights recorded (Table I). The testes of the animals injected with the hormone were not much inferior in weight to their respective controls. For those receiving up to 13.0γ of theelol per day the testis weight is over 90 per cent of that of the controls, while for those receiving 32.5γ the value is about 85 per cent. If we compare the last group with the average of all the controls the value would be raised to over 90 per cent. As stated earlier, the greatest effect on weight was observed after the injection of theelin. When 1.65γ of theelin were injected daily the testes were a little over 75 per cent of the control, while with 6.6γ the value decreased to about 67 per cent. It may be mentioned at this point that these figures indicate that the order of activity of these two hormones in producing these effects is comparable to their respective capacities to produce estrus.

The values recorded for the prostate and Cowper's glands follow in the same order. The weight of the pituitaries show no significant variation, the average for all of the animals being about 8 mg.

Because of a relatively wide variation in the individual weights of the testes, prostate and Cowper's glands it was thought desirable to subject the data to statistical treatment. The values: Difference Between the Means/ Probable Error of the Difference Between the Means were calculated for the structures listed above. The calculations for the weights of the testes, prostate and Cowper's glands for the total number of experimental and control animals are 5.2, 7.3 and 11.6, respectively, showing that the difference between the two groups is real and not due to chance.

At autopsy some of the animals which had not produced litters were examined for motile sperm from the epididymis. Nine controls and 16 injected animals were examined and motile sperm of normal appearance were observed in all cases.

A summary of part of the data on the injected females is given in Table II. One hundred matings were recorded in the group of 41 injected animals. Sixty-one pregnancies followed and 41 litters were produced. Progeny from 11 of the females survived and were weaned at the normal age. Fertility was tested in 4 of these litters by brother-sister matings and all were found normal.

It is important to mention that in another group a young male was included by mistake. All of these animals were injected with 13.0γ of theelol daily. After being injected for about 5 months this male had impregnated 6 of the 9 females. All 6 gave birth to litters which, however, failed to survive.

The poor survival of the litters is correlated with a disturbance in the ability of the mother to suckle the young. In at least 6 cases, of the 47 litters produced, there was definite evidence of poor or suspended lactation. If the young of these mothers were given to normal foster mothers, before the young rats became too weak, they survived and grew to be as

TABLE II

ADMINISTRATION OF THEELOL TO FEMALE RATS

Animal	Theelol Injected	Days Injected	Matings	Pregnancies	Litters Born	Litters Died	No. of Young in Litter	Day of Delivery	Lactation
63 B	0.65	143	1	1	1	0	6	23	
63 R	0.65	172	2	2	2	1	5, 9	23, 22	
63 y	0.65	316	3	1	0	—	—	—	
63 w	0.65	270	3	3	3	3	5, 6, 7	23, 21, 21	P, P, P
58 RI	3.25	196	3	2‡	2	1	5, 5	21, 23	?, P
58 R2	3.25	316	5	3‡	3	2	1+, 3+, 2	26, 22, 23	?, ?, P
60 R1	3.25	172	2	1‡	1	0	8	21	
60 R2	3.25	316	8	2‡	1	1	3+	23	
60 R3	3.25	173	2	2	2	1	2, 9	23, 24	
59 R1	6.5	159	1	1	1	0	3	23	
59 R2	6.5	231	3	3	3	2	4, ?, 6	23, 22 or 23, 23	P, ?, P
61 R1	6.5	316	7	5	3	3	1+, 1‡	24, 25, 25	?, ?, P
61 R2	6.5	177	2	2	1	0	5	?	
61 R3	6.5	254	4	0	—	—	—	—	
67 L1	6.5	260	0	—	—	—	—	—	
67 L2	6.5	260	0	—	—	—	—	—	
67 L3	6.5	260	2	0	—	—	—	—	
67 R1	6.5	260	1	1	0	—	—	—	
67 R2	6.5	260	1	0	—	—	—	—	
64 B	13.0	295	4	?	0	—	—	—	
64 R	13.0	195	4	2‡	1	0	3	22	P
64 y	13.0	295	5	1‡	1	1	1+	24	
64 W	13.0	210	3	2	2	1	4, 3	21, 22	
64 yb	13.0	295	8	4	1	1	2‡	23	
64 Rb	13.0	243	3‡	3	2	1	8, 7	23, 22	
68 LR1	13.0	260	4	0	—	—	—	—	
68 LR2	13.0	260	1	1	0	—	—	—	
68 R1	13.0	260	2	2	2	2	5, ?	?, ?	
68 L1	13.0	260	4	3	2	2	?, 4‡	?, 23	
68 R2	13.0	260	0	—	—	—	—	—	
68 L2	13.0	260	0	—	—	—	—	—	
68 R3	13.0	260	3	3	3	3	4, 5, 3+	?, 23, ?	
68 L3	13.0	260	3	3	2	2	?, ?	?, ?	
65	32.5	179	1	0	—	—	—	—	
65 R	32.5	179	0	—	—	—	—	—	
65 B	32.5	179	4	1	0	—	—	—	
65 y	32.5	179	0	—	—	—	—	—	
65 W	32.5	179	1	1	0	—	—	—	
65 BB	32.5	179	5	3	1	0	2	24	
65 Rb	32.5	179	1	1	0	—	—	—	
65 yb	32.5	179	3	2	1	0	7	?	
58 L1	Control	—	2	1‡	1	0	6	22	
58 L2	Control	—	2	1‡	1	0	9	21	
60 L1	Control	—	1	1	1	0	5	22	
60 L2	Control	—	1	1	1	0	7	22	
59 L1	Control	—	1	1	1	0	2	22	
59 L2	Control	—	1	1	1	0	3	22	
61 L2	Control	—	1	1	1	1	7	21	

‡Probably more than number given. +More than number given. P—Deficient or suspended secretion of milk.

strong and healthy as any normal animal. Conversely, when normal young rats were given to such poorly lactating females, the young grew poorly or even perished.

The records of two animals may be cited for illustration: 58 R I. 11-26-31. Litter of 5, 2 females dead and 3 males living when found. 12-3-31. One has died; other two have scarcely grown. 12-4-31. Two young males of 58 R I marked and given to foster mother, 58 R I F I, which has 4 of her own. 12-9-31. One of two males died but the other has doubled his weight since 12-4-31. 4-13-32. Now as large and strong as other normal animals.

63 W. 11-19-31. Litter of 5; look good. 11-20-31. Two males and one female left; look weak; have not nursed; given to foster mother but died because of weakened condition. 63 W remated and injected as before. 12-30-31. Litter born, 5 living and 1 dead; have not nursed; given to foster mother W F 3 and 5 of latter's young given to 63 W. 1-2-32, young given to 63 W all died. 1-5-32, young of 63 W which were given to W F 3 doing fine; 2 males and 2 females remaining. 63 W remated and injected as before. 3-1-32. Litter of 7, some milk in stomachs. 3-3-32. Four left; very weak; have not nursed; given to

foster mother, W F 4, but were dead next day. Young of W F 4 given to 63 W but they did not nurse, apparently due to weakened condition, and failed to survive.

Since these were preliminary experiments, it is thought that many of the other litters died from the same cause before the lactation factor was fully appreciated. In other cases poor lactation combined with delayed delivery was thought to have caused death. The mothers with but few exceptions attempted to care for the young whether they had milk or not. Some of the 36 litters which failed to survive were born dead but the percentage is not known.

Delayed delivery was another factor contributing to the poor survival of the litters. In at least 21 cases delivery was later than the 22nd day of gestation, counting from the appearance of sperm in the smears. In these litters born between the 22nd and the 26th day, survival was poorest. In 6 other cases laparotomy was performed when there was evidence of faulty delivery and fetuses in various stages of resorption were observed.

After the cessation of the injections some of the females were still capable of rearing young as shown by 6 which became pregnant and raised normal litters. These had been injected daily for 176, 168 and 324 days with 6.5, 3.75 and 3.75γ of theelol, respectively. The other 3 had been injected daily with 32.5γ for 179 days. However, a number of other animals under observation after the cessation of treatment failed to reproduce.

The injury to the ovary produced by short period treatment in the young animal appears to have been largely repaired after prolonged treatment. Since there were 67 pregnancies the ovaries now seemed to be capable of functioning in an approximately normal manner. Furthermore, the ovaries and uteri appeared normal in practically all animals examined at laparotomy. At the end of the experiment many of the animals were killed and these also appeared to have ovaries and uteri of normal appearance. Likewise no gross pathological changes were seen in any of the other organ systems.

Since one of the primary functions of the female sex hormone is the regulation of the estrus cycle it seemed desirable to determine the effect of continued treatment on this phase of reproduction uncomplicated by pregnancy. For this purpose estrus changes were followed by daily observations on the vaginal smear in a group of unmated normal females. The average length of the normal estrus cycle as determined on 16 of 18 normal animals was 4.6 days; the cycles of the other two were irregular. The injection of theelol in doses of from 0.65 to 13.0γ daily for 60 days in 10 animals gave an average cycle of 4.9 days. Eight other animals receiving the same treatment ran cycles with periods of prolonged estrus or diestrus of 5 to 25 days interspaced with periods of regular cycles. Twelve animals injected for 70 days longer, or a total of 130 days, continued to run cycles which were somewhat irregular. Smears were continued for 108 days after the cessation of injection, on 2 animals which had been injected daily for 130 days with 0.65γ theelol. One of these animals continued to run a

fairly normal cycle of 4 to 5 days' duration while the other was more irregular.

In the group of spayed rats the daily injection of from 0.65 to 13.0γ of theelol for 180 days was characterized by the production of irregular cycles of 5 to 10 days' duration interspaced with long periods of estrus and diestrus up to over 30 days. These females were mated with normal males over a period of 4 months but no copulations were recorded. During the last 2 months the dosage was increased five-fold without producing any pronounced change in response.

GROWTH

During the course of the experiment outlined above changes in the body weight of the male animals were recorded at 5-day intervals. The character of the growth curves of the injected and control animals is essentially the same and the final weight attained by the animals may be taken as a fair index of the effect on growth. The difference in growth response in the two groups usually became apparent in from 10 to 20 days. The average values for both groups for the different injections are given in Table I. When one compares the average gain in weight of the hormone-injected and the control animals it is seen that the former, as a group, are about 13 per cent below the controls. From the data presented it appears that the animals receiving 32.5γ of theelol daily were not much inferior to those receiving the smaller doses. The animals receiving 6.6γ of theelin were most inferior, averaging about 75 per cent of the controls. These figures are suggestive but must not be taken as conclusive. These data were also subjected to statistical treatment. The Difference Between the Means/Probable Error of the Difference Between the Means was calculated using the total number of experimental and control animals. The resulting value of 3.37 shows that the difference is significant. To give a true comparison between dosage levels and between theelin and theelol in producing these effects requires a much more extensive investigation. Less extensive observations on the females indicate a similar response.

DISCUSSION

The data presented show that both male and female rats can pass through the normal series of events concomitant with reproduction after having been constantly under the influence of large doses of the estrogenic hormone from the early age of weaning. Although Table I does not bring out clearly the relative fertility of the two groups of male rats, the hormone-injected animals are somewhat inferior to their controls. However, since motile sperm of normal appearance were observed at autopsy in all of the animals examined which had not mated, one may conclude that the treatment did not interfere greatly with spermatogenesis. This does not mean necessarily that the sperm were fully normal and capable of fertilizing the ova. A detailed study of sperm morphology, which is correlated with fertility (Moench and Holt, 1931) was not attempted. The

density of sperm suspension and the amount of testicular material are not important (Hutt, 1929). Excluding one animal, 57 R I, 64 per cent of the matings of the injected animals were fertile. In the case of animal 57 R I just noted there were 12 recorded matings with but two litters produced. Since, according to some writers about 90 per cent of the matings of normal animals are fertile, it seems that although our animals were capable of impregnating the female their fertility was subnormal. The failure of a number of animals to mate is not easily explained, but since nearly 28 per cent of the injected males failed to mate and since apparently normal sperm were found in all of these which were examined, it is possible that other factors are involved in the mating response.

Though earlier and less extended experiments from different laboratories have clearly demonstrated that the administration of the estrogenic hormone is injurious to genital development, it is evident from the data presented in this paper that serious injury to the genital systems did not persist as treatment was continued. The greatest effect on the testis weight was produced by the injection of 6.6γ of theelin daily. In this case the testis weight was about 70 per cent of the normal. Fertility, however, was greater in this group than in any of the others.

A similar set of conditions was found in the females where there was no macroscopic evidence that ovarian development and function had been affected to any appreciable extent. The chief deleterious effects in the female occurred at a late period centering about the processes of delivery and lactation. As stated earlier, parturition was frequently delayed and sometimes accompanied with resorption of the fetuses. Some of the young were born dead but the percentage is not known. In these cases, the young though dead appeared normal, indicating that death was of recent occurrence. In a very few cases abnormally large fetuses were observed, indicating that intrauterine growth had progressed past the normal condition. In cases of delayed delivery death was probably due to retention too long within the uterus.

In our experiments the effects of the ovarian hormone on the general course of pregnancy appeared to be slight. The various publications on this subject which have appeared are not in close agreement. Some have reported no effect on gestation, while others have reported the termination of pregnancy. At present it is very difficult to evaluate satisfactorily the reports from different laboratories since the rat unit of one group may vary considerably from that of another. From the recent report on the histological changes produced by estrin in the pregnant uterus (D'Amour and Gustavson, 1934), it appears that under certain conditions the ovarian hormone will terminate pregnancy. The slight effect on gestation in our experiments even after the administration of large doses was probably due to the fact that the treatment was extended over long periods, giving time for adjustment to altered conditions on the part of the animal. To what extent the organism can undergo acclimatization to such agents remains to be determined.

The adverse effects on lactation with the resulting poor development or death of the young may possibly be attributed to a disturbance in the activity of the anterior hypophysis. The presence in the pituitary of a factor necessary for lactation has been demonstrated by Corner (1930), Turner, et al. (1931, 1933), Riddle, et al. (1932, 1933), Selye, Collip and Thomson (1933a, b), Catchpole, et al. (1933), Evans (1933) and others. The removal of the hypophysis in rats (Selye, Collip and Thomson, 1933b) or the administration of the estrogenic hormone to rabbits (Smith and Smith, 1933) and guinea pigs (Nelson, 1933) has been found to inhibit lactation. In our experiments the theelol administered appears to have either decreased the production of the lactogenic factor below the required level or to have impaired in some other way the capacity of the animal to produce milk.

The effects on the various phases of reproduction and growth are in agreement with the theory of Moore and Price (1932). According to this concept the primary sex hormone suppresses the activity of the hypophysis to a greater or lesser degree with the result that certain normal functions of that gland are impaired or suspended. With prolonged treatment our results have shown that impairment of certain functions may become less pronounced and that normal physiological function may become established. Subsequent experiments must determine to what extent the activity of the hypophysis may be controlled by hormonal methods.

Concerning the effect on estrus it is somewhat surprising that even with high doses of theelol the rhythmic response is still obtained in normal and spayed animals. Since the quantity of hormone administered was kept constant it would appear that there is a rhythmic response in the cells sensitive to the hormone. The factors responsible for this rhythmicity are not known. In the experiments presented in this paper mating was not observed in the spayed animals as reported by Allen, Doisy, et al. (1924). The dosage level may be important (Marrian and Parkes, 1930), but since some of the animals were receiving up to 65.0γ of theelol daily it would appear that other factors may be involved.

Our data leave a number of questions unanswered and additional information is being sought on the effect of various dosage levels and on the relation of theelin to theelol in producing the effects presented in this paper.

SUMMARY

Groups of male rats were injected daily from weaning with quantities of theelol ranging from 0.65 to 32.5γ; of theelin from 1.65 to 6.6γ. The periods of injection ranged from 113 to 242 days. Of 53 male rats injected 24 successfully impregnated normal females; of 39 control males 17 were successful in siring litters.

The body weight of the injected males averaged 87 per cent of the body weight of the control males. The weights of the testes, prostates and Cowper's glands of the injected rats were less than those of the controls.

Forty-one female rats were injected daily from the time of weaning with quantities of theelol for the separate groups ranging from 0.65 to 32.5γ. The injections extended over periods of 143 to 316 days. During this time, 100 matings, 61 pregnancies and 41 litters occurred. Reproduction was below normal and an interference with lactation was observed.

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REFERENCES

Allanson, M.: J. Exper. Biol. **8**: 389. 1931.
Allen, E. and E. A. Doisy: Am. J. Physiol. **69**: 577. 1924.
Allen, E., E. A. Doisy, B. F. Francis, H. V. Gibson, L. L. Robertson, C. E. Colgate, W. B. Kountz and C. G. Johnson: Am. J. Anat. **34**: 133. 1924.
Allen, E., B. F. Francis and O. W. Craig: Anat. Rec. **29**: 379. 1925.
Bialet-Laprida, Z.: Compt. rend. Soc. de biol. **114**: 377. 1933a.
———————: Compt. rend. Soc. de biol. **114**: 381. 1933b.
Borchardt, E., E. Dingemanse, S. E. deJongh and E. Laqueur: Ztschr. f. d. ges. exper. med. **68**: 86. 1929.
Brouha, A.: Compt. rend Soc. de biol. **110**: 1023. 1932.
Brouha, L. and H. Simonnet: Compt. rend Soc. de biol. **93**: 557. 1925.
del Castillo, E. B.: Rev. Soc. argent. de biol. **8**: 591. 1932.
Catchpole, H. R., W. R. Lyons and W. M. Regan: Proc Soc. Exper. Biol. & Med. **31**: 301. 1933.
Corner, G. W.: Am. J. Physiol. **95**: 43. 1930.
Courrier, R.: Compt. rend. Acad. de sc. **179**: 2192. 1924.
Courrier, R. and G. Gros: Compt. rend. Soc. de biol. **110**: 1021. 1932.
D'Amour, F. E. and R. G. Gustavson: J. Pharmacol. & Exper. Therap. **51**: 353. 1934.
D'Amour, F. E., M. C. D'Amour and R. G. Gustavson: J. Pharmacol. & Exper. Therap. **49**: 146. 1933.
Doisy, E. A., J. Curtis and W. D. Collier: Proc. Soc. Exper. Biol. & Med. **28**: 885. 1931.
Evans, E. I.: Proc. Soc. Exper. Biol. & Med. **30**: 1372. 1933.
Fellner, O. O.: Pflüger's Arch. f. ges. Physiol. **189**: 199. 1921.
Gardner, W. V. and C. W. Turner: Mo. Agr. Exp. Sta. Res. Bul. 196. 1933.
Gostimirovic, D.: Biol. Zentralbl. **50**: 599. 1930.
Gould, V. L. and E. A. Doisy: Unpublished Observations. 1924.
Haberlandt, L.: Pflüger's Arch. f. ges. Physiol. **202**: 1. 1924.
———————: Klin. Wchnschr. **41**: 553. 1928.
———————: München med. Wchnschr. **77**: 2064. 1930.
Hauptstein, P.: Endokrinologie, **8**: 169. 1931.
Herrmann, E. and M. Stein: Wien. klin. Wchnschr. **29**: 177. 1916.
Hutt, F. B.: Proc. Roy. Soc. Edinburgh, **49**: 102. 1929.
Katzman, P. A.: Proc. Soc. Exper. Biol. & Med. **29**: 700. 1932.
Kelly, G. L.: Surg. Gynec. & Obst. **52**: 713. 1931.
Kunde, M. M., F. E. D'Amour, R. G. Gustavson and A. J. Carlson: Am. J. Physiol. **96**: 677. 1931.
Laqueur, E. and S. E. deJongh: J. A. M. A. **91**: 1169. 1928.
Leonard, S. L., R. K. Meyer and F. L. Hisaw: Endocrinology, **15**: 17. 1931.
Levin, L., P. A. Katzman and E. A. Doisy: Endocrinology, **15**: 207. 1931.
Loeb, L. and W. B. Kountz: Am. J. Physiol. **84**: 283. 1928.
Marrian, G. F. and A. S. Parkes: J. Physiol. **69**: 372. 1930.
Moench, G. L. and H. Holt: Am. J. Obst. & Gynec. **22**: 199. 1931.
Moore, C. R. and D. Price: Am. J. Anat. **50**: 13. 1932.
Nelson, W. O.: Proc. Soc. Exper. Biol. & Med. **30**: 953. 1933.
Parkes, A. S. and C. W. Bellerby: J. Physiol. **62**: 145. 1926.
———————: J. Physiol. **62**: 301. 1927.
Pincus, G. and N. Werthessen: Am. J. Physiol. **103**: 631. 1933.
Reiprich, W.: München. med. Wchnschr. **78**: 343. 1931.
Riddle, O., R. W. Bates and S. W. Dykshorn: Anat. Rec. **54**: Suppl. 25. 1932.
———————: Proc. Soc. Exper. Biol. & Med. **30**: 794. 1933.
Selye, H., J. B. Collip and D. L. Thomson: Proc. Soc. Exper. Biol. & Med. **30**: 588. 1933a.
———————: Proc. Soc. Exper. & Med. **30**: 589. 1933b.
Smith, G. van S. and O. W. Smith: Am. J. Physiol. **103**: 356. 1933.
Smith, M. G.: Bull. Johns Hopkins Hosp. **39**: 203. 1926.
Spencer, J., R. G. Gustavson and F. E. D'Amour: Proc. Soc. Exper. Biol. & Med. **28**: 500. 1931.

Spencer, J., F. E. D'Amour and R. G. Gustavson: Am. J. Anat. **50**: 129. 1932.
————————: Endocrinology, **16**: 647. 1932.
Steinach, E. and H. Kun: Biol. generalis, **2**: 815. 1926.
Turner, C. W.: Mo. Agr. Exp. Sta. Res. Bul. 156. 1931.
Turner, C. W. and W. U. Gardner: Mo. Agr. Exp. Sta. Res. Bul. 158. 1931.
Turner, C. W. and A. B. Schultze: Mo. Agr. Exp. Sta. Res. Bul. 157. 1931.
Wade, N. J. and E. A. Doisy: Proc. Soc. Exper. Biol. & Med. **28**: 714. 1931.

RELATIONSHIP OF THE HYPOPHYSIS TO HAIR GROWTH IN THE ALBINO RAT†

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INTRODUCTION

Changes in the character of the skin and changes in the growth and distribution of hair long have been associated clinically with endocrine disturbances, particularly those involving the thyroid, hypophysis and adrenals.

In recent times pituitary therapy has been advocated in the treatment of certain types of baldness. Bengtson (1, a, b) has used anterior pituitary extracts clinically with reported success in alopecia areata and alopecia totalis. He has tried a variety of other endocrine preparations and local agents in these conditions with no success. Kohn (2) reported favorably on the use of pituitary therapy in premature baldness, but the results of Lord (3) were negative in this condition. Stanley and Hawkins (4) obtained suggestive results in alopecia in prisoners following pituitary implants.

Controlled animal experimental studies have shown a definite relationship between the thyroid and the dermal system (5, a, b) but observations relating the cutaneous structures to the hypophysis have been noted only incidentally. Cushing (1912) (6) found that the skin of hypophysectomized dogs became dense, dry, and less movable than normal and that the hair tended to fall. Smith (7, a, b) noted that the hair of hypophysectomized rats retained its fine, infantile character; pituitary implants caused the hair of these animals to assume the adult appearance. Thompson and Gaiser (8) observed that similar changes in the hair coat of hypophysectomized rats could be produced by the injection of growth extracts of the pituitary. White (9) observed a partial shedding of the hair in hypophysectomized rabbits. Evans (10) reported that daily injection of growth hormone into normal shepherd dogs caused the hair coat to become long, thick, soft, and glossy.

As no study has been made to demonstrate the quantitative relationship of the hypophysis to hair growth the following experiment was planned: Twenty-one rats were completely hypophysectomized. Twelve of these received no replacement therapy; nine were injected daily with Phyone. The two groups were compared with one another and with normal rats of the same age and size in respect to (a) rate of hair growth, (b) microscopic size of the roots and shafts of the hairs, (c) weight of hair per square centimeter of body surface and (d) microscopic structure of the skin. The study also included a complete necropsy.

METHODS

Young, male, albino rats were used. Hypophysectomy was performed, using the technique of Thompson (11) with minor modifications. Growth stasis for a period of 30 days following the operation was used as a working criterion of complete removal. Careful gross examination and microscopic sections obtained at necropsy gave additional information concerning the completeness of the hypophysectomy.

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All the animals were kept in clean wire cages. The temperature of the laboratory was kept between 75° and 80° F. All of the rats received Baby Chick Starter* as the basic dietary mixture. In addition, a liberal amount of fresh milk and whole wheat bread was fed daily, and fresh lettuce was given three times a week.

The growth hormone extract (Phyone) used in this investigation was prepared according to the method of Van Dyke and Wallen-Lawrence (12) from beef and sheep pituitaries. The final extract was made of such a concentration that each cubic centimeter represented ½ to ⅔ gram of fresh anterior lobe. The extract was sterilized by Seitz filtration, sealed in sterile ampoules and stored in the refrigerator. Daily subcutaneous or intraperitoneal injections of 0.5 cc. were used. This preparation was found to be quite potent in producing growth of hypophysectomized rats.

In order to determine the rate of growth of the rat's hair, a method was adopted similar to that used by Chang (5, a). Rectangular areas about two by five centimeters were cleanly shaved across the back, in a saddle position. Normal, control rats were shaved when they weighed about 100 grams; the hypophysectomized rats were shaved two weeks after operation. The hypophysectomized rats given Phyone were shaved about two weeks after the injections were started. The rats were carefully examined at three-day intervals and the number of days required for the growth of the initial 2 mm. coat was determined. Subsequently, at each reading several hairs (6 to 8) were removed and carefully measured with a caliper rule. The time required for the coat to become fully developed was determined by noting the number of days required for the hair to reach full length and the shaved area to become indistinguishable.

The length and diameter of the root and the maximum diameter of the shaft of hair from each animal was measured with the aid of a micrometer. Owing to the oval shape it was found necessary to measure each hair on a separate slide, and to flatten each hair individually with a heavy cover slip.

At the end of the experiment the hair was collected for weighing, for which the method of Lightbody and Lewis (13) was employed. The pelt was removed immediately after the animal was killed. The skin was cut on a medial line of the ventral surface, about the legs at the tarsal bones and about the neck on a line immediately posterior to the ears. The pelts were scraped free from as much subcutaneous tissue as possible. Each pelt was then immersed in a liter of phosphate solution (pH 6.8) containing one gram commercial pancreatin. After incubation at 37° C. for 24 hours the pelts were removed from the digestion mixture and the hair which had become loosened was separated from the other tissue. The adherent tissue was removed by suspending the hair in distilled water; the mixture was stirred and filtered through copper gauze (60 mesh). The hair was then transferred to extraction thimbles and dried at 60° C. The thimbles were then transferred to an extraction apparatus and extracted successively with alcohol, ether and distilled water. The thimbles were again dried at 60° C. and stored in a desiccator over calcium chloride. The cleaned and dried hair of each animal was carefully weighed to three decimal places.

In order to determine the weight of hair per unit of surface area, a modification of the surface area formula of Meeh-Rubner was employed, containing Diack's (14) value for K and the nutritive corrective factor of Cowgill and Drabkin (15) and Lee (16).*

At necropsy, small pieces of skin were taken for microscopic study. They were fixed in formalin and stained with hematoxylin and eosin.

RESULTS

The following general data pertain to the age, weight, and duration of life of the three main groups of experimental animals: (a) Hypophysectomized rats. This group consisted of 12 completely hypophysectomized animals. At the time of operation they averaged 137 grams in weight. They lived for an average of 94.7 days; eight died and four were sacrificed. Their average weight at death was 109 grams, and they were 5.3 months old. (b) Hypophysectomized rats given Phyone. This group con-

*Chick starter, made by a local firm contains white bran, wheat middlings, yellow corn meal, certified cod liver oil, calcium and salt.

*Surface area $= 7.47\ W^{2/3}\ \frac{0.310}{N}$; where $N = \frac{W^{1/3}}{L}$.

sisted of 9 completely hypophysectomized animals. At the time of operation they averaged 134 grams in weight. Twenty-eight days after operation they averaged 119 grams. At this time daily injections of 0.5 cc. of Phyone were started, and these were continued for 51 days. Their average total age was 4.7 months, and their final weight was 253 grams. This represented an average gain of 134 grams for each animal (2.6 grams per day) while they were receiving the growth hormone. (c) Control Rats. Three groups of normal controls were used. First, a series of three rats, 1.5 months of age and averaging 117 grams in weight, served as a weight control for the hypophysectomized rats. This was also a weight control group for the hypophysectomized, injected animals at the time of their operation. Second, a group of four young rats was used to determine the normal rate of hair growth for growing rats. These increased in weight from 100 to 223 grams and were 3 months of age when they were sacrificed. Third, a group of six animals, 5 months of age and averaging 298 grams in weight, served as an age control group for both the non-injected and hypophysectomized, injected groups, and as an approximate weight control group for the latter.

Rate of Hair Growth. The hypophysectomized rats grew hair at a slower rate than young normal rats. The hypophysectomized rats which received Phyone showed a slightly faster rate of hair growth than did the normal controls. This was true both for the growth of the initial 2 mm. coat and for the development of the full coat. (Table 1.)

TABLE 1

RATE OF HAIR GROWTH

Expressed as Number of Days Required for Development of Coat

	Normal Rats	Hypophysectomized Rats	Hypophysectomized Rats Plus Phyone
Number of days for growth of 2 mm. coat	10.5	14.0	9.2
Number of days for growth of full coat	28.5	33.3+	26.7
Number of rats	4	12	9

Microscopic Size of Hair. The length and diameter of the roots as well as the maximum diameter of the shafts of the hairs removed from the hypophysectomized rats about 90 days after operation were found to be considerably smaller than corresponding measurements of normal rats' hair. The length and diameter of the roots of the hypophysectomized group injected with Phyone were very slightly less than those of the normals, but the maximum diameter of the shafts were the same in both groups. Comparing samples removed 28 and 90 days following hypophysectomy, it was noted that while the roots were of approximately the same size, the maximum diameter of the shafts was considerably larger in the 28-day group. (Table 2 and Figs. 1, 2, and 3.) This was probably due

to the fact that these larger hairs had developed previous to the operation and had been retained for this period of time. That this was the case was evidenced by the characteristic pattern of these hairs. The wide portion of the shaft was near the terminal end, while there was a long thin shank next to the atrophied root.

TABLE 2

MICROSCOPIC SIZE OF ROOTS AND SHAFTS OF HAIRS

Figures given are averages for the groups in micra

Groups	Number of Animals	Diameter of Root	Length of Root	Maximum Diameter of Shaft
Normals	5	77	212	144
94 days hypophysectomized	10	38	124	84
28 days hypophysectomized	9	44	127	123
28 days hypophysectomized+Phyone (14 days)	9	68	202	145

Weight of the Hair Coat. The total weight of the hair coat of the hypophysectomized rats was slightly more than that of immature rats of approximately the same weight, but much less than that of normal rats of the same age. The weight of the hair of the hypophysectomized, injected rats was about three times that of the operated rats not receiving Phyone, though they only doubled in weight. Their coat was heavier than that of normal rats of slightly greater body weight. (Table 3.)

TABLE 3

WEIGHT OF HAIR COAT

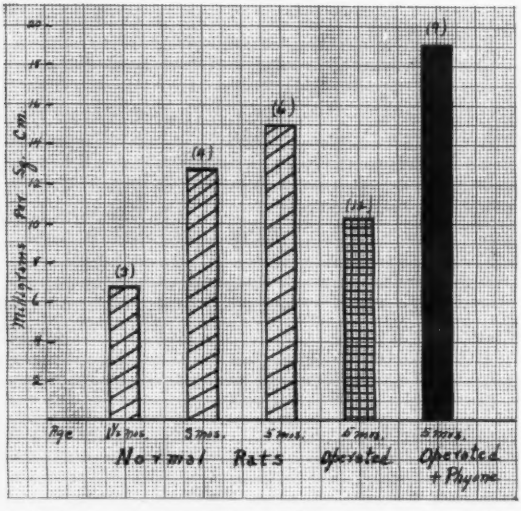
	Weight of Hair Coat				
	Normal Control Rats			Hypophysectomized Rats	
Age	1.5 Months	3 Months	5 Months	No Treatment 5 Months	Plus Phyone 5 Months
Number of rats	3	4	6	12	9
Length (nose-anus) in centimeters	16.4	18.6	21.2	16.3	20.2
Body weight (grams)	117.	223.	298.	109.	253.
Weight of hair coat in grams	1.266	3.498	4.863	1.878	5.672
Surface area, sq. cm.	185.8	273.5	324.6	180.5	295.8
Weight of hair per sq. cm. body surface in milligrams	6.81	12.79	14.98	10.33	19.17

The figures given are averages for each group.

The weight of hair per square centimeter body surface of the hypophysectomized animals was more than that of their normal weight controls having immature coats, but considerably less than that of their normal age controls. The weight of hair per square centimeter of the hypophysectomized rats which received Phyone was almost twice that of those which

did not receive injections, and even greater than that of normal rats of the same age and weight. (Table 3 and Graph 1.) The weight of hair per square centimeter was considered to represent most accurately the true condition of the hair coat.

Graph 1



Average weight of hair coat, expressed as milligrams per square centimeter body surface.

Microscopic study of the skin of the hypophysectomized animals brought out certain significant differences from the normal. The skin of the hypophysectomized rats was definitely thinner than normal. After staining, the thickness was measured from the attachment of the corneum of the epidermis down to the junction of the cutis and the platysmal muscle. This thickness averaged 1.21 mm. in the normal age control group; 1.03 mm. for the normal weight control group; 0.72 mm. in the case of the hypophysectomized rats; and 1.07 mm. for the injected rats. (Plate I, Figs. 4, 5, and 6.) No essential changes were noted in the epidermis. The marked thinning of the dermis in the group of hypophysectomized rats seemed to be due chiefly to a decrease in the amount of collagenous tissue. The individual connective tissue fibers were smaller than those of the normal skin. The hair follicles and papillae appeared smaller in the hypophysectomized group, but closer together due to the small amount of intervening tissue. There seemed to be an increased amount of fat cells in the lower portion of the dermis. (Plate I, Fig. 8.) The hypophysectomized, injected group showed an increase in the amount of collagenous tissue and the normal relationships of structure were restored. In this group a characteristic type of young connective tissue was found surrounding many of the bulbs of the hair follicles. (Plate I, Fig. 7.)

PLATE I

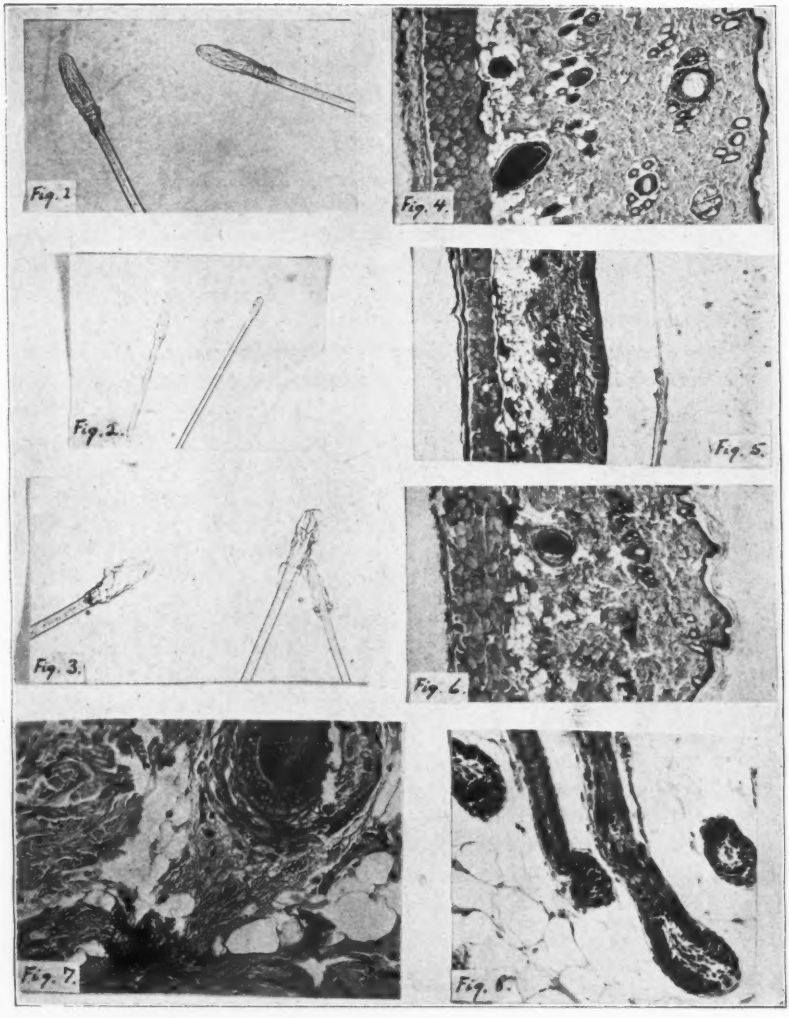


Figure 1. Microscopic appearance of roots of hairs removed from back of normal rat. x30.

Figure 2. Roots of hairs from hypophysectomized rat. x30.

Figure 3. Roots of hairs from hypophysectomized rat injected with Phyone. x30.

Figure 4. Microscopic appearance of skin from back of normal rat. x50.

Figure 5. Skin from back of hypophysectomized rat. Compare thickness and structure with normal above. x50.

Figure 6. Skin from back of hypophysectomized rat injected with Phyone. x50.

Figure 7. Skin of hypophysectomized rat after injection with Phyone. Shows the characteristic type of young connective tissue surrounding the hair follicle and streaming down to the left. x140.

Figure 8. Skin of hypophysectomized rat. Shows the structure of the hair follicles and the increased amount of fat present. x140.

DISCUSSION

The syndrome produced by complete removal of the pituitary gland in rats was found to correspond closely to that described by Smith (7), Thompson (11), and Evans (10). The nine young rats hypophysectomized at about 120 grams in weight showed remarkably few immediate ill-effects. After an initial loss of weight, due probably to the operative procedure itself and to some difficulty in eating for 2 or 3 days, they remained at practically a constant weight for a period up to 4 months. Their coats did not develop normally but remained immature, being rather fine, somewhat thin and shaggy, and the hair could be pulled out easily. The testes decreased in size and usually could not be palpated. The hypophysectomized animals continued to resemble young immature rats. These rats later developed varying degrees of cachexia. Five died and four survived the experimental period of 4 months. The cachexia was manifested by a characteristic posture, the rat sitting with a bowed back, by the emaciation which developed, and by the fact that the rat felt cool. These rats were very quiet and slept most of the time. However, when they were handled they would be quite alert and tense. Most of them developed warts on the nose, ears and tail. The hair became especially thin at the base of the tail. The three older rats operated at 220 grams showed a much more marked loss of weight and developed cachexia early. All three of these rats died within 80 days following the operation.

Uniform relief from the hypophysioprivous syndrome followed the administration of the growth hormone extract, except in two cases which were in extremis when it was begun. Growth was resumed and a rapid and uniform change in the constitutional state of these animals resulted. This was indicated by a return of muscular strength and tone, giving them a normal feeling of plumpness and firmness. The coat soon became normal in appearance. These hypophysectomized, injected rats could not be distinguished from normal rats, except by examining the testes, which remained small and soft. Toward the end of the injection period an increased folding of the skin was noticed. This appeared to be due to a true hypertrophy of the skin and subcutaneous tissues.

The question arises as to whether the stimulation of hair growth is a specific effect of the growth-promoting hormone. This was not tested directly, but it was noted that the injection of Phyone into hypophysectomized rats produced a greater relative increase in hair weight than in general body weight. The hair tripled in weight during the time in which the body weight doubled. This suggests a specific stimulation of the hair follicles by the growth hormone.

The observation that the hair coat of the hypophysectomized, injected rats weighed more than that of normal rats was explained as being due at least in part to the increased amount of skin. This was evidenced by the excessive folding and wrinkling of the skin noticed toward the end of the experimental period.

It was not determined whether Phyone would stimulate normal rats to greater hair growth. However, that this would probably occur was evidenced in the case of an incompletely hypophysectomized rat. This rat (No. 131) was operated but continued slowly to gain weight. It reached a "plateau" early at about 200 grams. After this it received Phyone daily for a period of 3 months, during which it increased in weight to 360 grams. This rat was found to have the heaviest coat of hair of any rat sacrificed; it weighed 7.707 grams. At necropsy about 1/5 of the pituitary gland was found to be present.

A conservative attitude should be maintained in applying the present findings on hair growth to clinical therapy. A very profound endocrine disturbance, producing far-reaching metabolic changes, was produced in the rats by complete removal of the pituitary. The hypophysectomized rats had a hair coat, although it was definitely distinguishable from the normal; furthermore, they grew new hair on shaved areas, though it occurred at a somewhat slower rate than normal. The pituitary is then by no means the sole factor concerned in hair growth, though it does seem to have a definite effect on it. Exhibition of the hypophyseal growth hormone in the treatment of the common type of seborrheic alopecia in which there is usually no constitutional evidence of pituitary dysfunction would be illogical. In this condition there are sufficient local pathological changes in the scalp to account for the baldness. On the other hand, in those cases of alopecia areata or totalis, especially in young individuals where the alopecia is accompanied by other manifestations of endocrine disturbance, pituitary therapy with potent preparations of the growth autocoid given hypodermically is considered to be indicated.

SUMMARY

1. The average time required for the growth of a full hair coat on shaved areas in a group of 12 hypophysectomized rats was 33.3 days; similar hair growth was attained in a group of 4 normal animals of the same age in 28.5 days. Daily injection of the growth-promoting extract, Phyone, restored the rate of hair growth of the hypophysectomized animals to normal.

2. The microscopic size of the roots and shafts of hair from the hypophysectomized rats was markedly smaller than that of hair from normal rats. The roots and shafts of the hair from the hypophysectomized rats injected with Phyone were of practically normal size.

3. The weight of hair of the hypophysectomized rats (per square centimeter body surface) was 70 per cent that of the normal; that of the hypophysectomized, injected rats was 127 per cent the normal.

4. Histological evidence of atrophy of the skin was observed in the hypophysectomized rats; the histological picture of the skin was almost completely restored to normal by the administration of pituitary growth extract, and a characteristic type of young connective tissue was found surrounding the hair follicles.

5. The conclusion is reached that the pituitary gland exerts a decided influence on the growth of hair in the albino rat.

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REFERENCES

1. Bengston, B. N.: (a) Pituitary therapy of alopecia, a preliminary report. J. A. M. A. **97:** 1355. 1931.
   (b) Various endocrine extracts and local therapies in alopecia. Clin. Med. & Surg. **41:** 76. 1934.
2. Kohn, T.: Preliminary report on the use of anterior pituitary substance in common baldness. Virginia M. Monthly, **59:** 425. 1932.
3. Lord, L. W.: Anterior lobe pituitary extract in the treatment of alopecia. Arch. Dermat. Syph. **28:** 381. 1933.
4. Stanley, Leo L. and J. O. Hawkins: Implantations of anterior pituitary glands in alopecia. M. J. & Rec. **137:** 462. 1933.
5. (a) Chang, Hsi Chung: Specific influence of the thyroid gland on hair growth. Am. J. Physiol. **77:** 562. 1926.
   (b) Chang, Hsi Chung and Teh Pei Feng: Further studies on thyroid and hair growth. Chinese J. Physiol. **3:** 57. 1929.
6. Cushing, H.: The pituitary body and its disorders. J. B. Lippincott Co., Philadelphia, 1912.
7. (a) Smith, P. E.: The disabilities caused by hypophysectomy and their repair. J. A. M. A. **88:** 158. 1927.
   (b) Smith, P. E.: Hypophysectomy and a replacement therapy in the rat. Am. J. Anat. **45:** 205. 1930.
8. Thompson, K. W. and D. W. Gaiser: The effect of diet and pituitary growth hormone on hypophysectomized rats. Yale J. Biol. & Med. **4:** 677. 1932.
9. White, W. E.: Effect of hypophysectomy in the rabbit. Proc. Roy. Soc. B**114:** 64. 1933.
10. Evans, H. M., K. Meyer and M. E. Simpson: The growth and gonad-stimulating hormones of the anterior hypophysis. Memoirs Univ. of Cal. Vol. 11, 1933.
11. Thompson, K. W.: A technique for hypophysectomy of the rat. Endocrinology, **16:** 257. 1932.
12. Van Dyke, H. B. and Z. Wallen-Lawrence: On the growth-promoting hormone of the pituitary body. J. Pharmacol. & Exper. Therap. **40:** 413. 1930.
13. Lightbody, H. D. and H. B. Lewis: Metabolism of sulfur; the relation of protein and cystine content of diet to growth of hair in white rat. J. Biol. Chem. **82:** 485. 1929.
14. Diack, S. A.: The determination of the surface area of the white rat. J. Nutrition, **3:** 289. 1930.
15. Cowgill, G. R. and D. L. Drabkin: Determination of a formula for the surface area of the dog together with a consideration of formulae available for other species. Am. J. Physiol. **81:** 36. 1927.
16. Lee, M. O.: Determination of the surface area of the white rat with its application to the expression of metabolic results. Am. J. Physiol. **89:** 24. 1929.

ATTEMPTS TO REDUCE THE SYMPTOMS OF EXPERIMENTAL DIABETES BY IRRADIATION OF THE HYPOPHYSIS

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According to the prevailing theory of diabetes mellitus, the disturbance of carbohydrate metabolism is due to an insufficiency of the endocrine secretion of the pancreas. That other factors may be involved has been suggested by: (1) the failure to find typical and constant lesions exclusively characteristic of the disease in many, if not most, fatal cases of diabetes (Anderson, Boyd, Joslin); (2) the observation that ninety per cent of the pancreas of experimental animals must be removed or destroyed before severe symptoms of diabetes are produced (Macleod); (3) that extracts of the pancreas in fatal cases have shown the presence of insulin in amounts that would (theoretically) assure life for several weeks (Epstein).

These and other facts have suggested that other factors may play a rôle in the production of the disease. Hyper-function of the adrenal medulla has been considered by some workers as a causative factor, and Gordon cites numerous references indicating that irradiation of the adrenals has led to a marked improvement in diabetes. The part played by the hypophysis seems to be more important, as is evident from the following clinical and experimental evidence.

As early as 1884, Loeb observed the occurrence of diabetes in some cases of acromegaly. Pierre Marie confirmed this observation several years later (1886), and since then Borchardt, Davidoff and Cushing, Rowntree and others have reported coincidental diabetes in twenty-five per cent or more of their cases. Davidoff and Cushing, viewing their clinical and anatomical observations in acromegaly, believe that the co-existent diabetes is due to hyperfunction of the acidophil cells of the anterior lobe. That the basophil cells may also exert a diabetogenic action is indicated by the observations of Cushing, who found hyperglycemia and glycosuria in cases of pituitary basophilism. Partial extirpation of the adenomatous anterior lobe diminishes the hyperglycemia in most of these cases and increases the tolerance for glucose. Clinical observations have likewise indicated that deficiency of the hypophysis (hypopituitarism) is usually accompanied by an increase in glucose tolerance.

Cushing (1911) was the first to observe experimentally that hypophysectomized dogs have an increased tolerance for glucose. Recently, Houssay and his associates in South America have reported detailed investigations on the effect of hypophysectomy in both normal and depancreatized animals. These workers find that in normal animals the removal of the hypophysis leads to: (1) a low fasting blood sugar which reaches convulsing levels during fasting; (2) a marked sensitivity to insulin which produces fatal convulsions in doses as small as 0.5 units per kilo; and (3) an increased tolerance to both ingested and injected glucose. These observations have been amply confirmed by Geiling and Britton, Corkill, Lucke, and others.

In completely depancreatized dogs in which the symptoms of diabetes are severe, hypophysectomy greatly diminishes the intensity of the disease and prolongs the life of the animal. Thus hypophysectomized depancreatized dogs survive for eight months or more, while depancreatized controls not receiving insulin succumb within one or two weeks. In these animals hyperglycemia, glycosuria,

azoturia, ketonuria, and polyuria are decreased; the tolerance for glucose is increased; the respiratory quotient, which is low during fasting (0.64-0.75), is raised—indicating that glucose is utilized in these animals. Most of these findings have been confirmed by Barnes and Regan, Soskin, and Kutz, et al.

Converse changes to those noted after hypophysectomy are brought about by the injection of suitable extracts of the anterior lobe into normal animals. Thus Houssay and Biasotti, Evans, et al., found hyperglycemia and glycosuria resulted; Baumann and Marine observed lipemia and polyuria; and Anselmino and Hoffmann found acetonemia. Although the exact röle of the hypophysis in carbohydrate metabolism is still obscure, it appears that the gland secretes a hormone which antagonizes insulin, and which stimulates gluconeogenesis. In doing this, it may act with, or be activated by, other endocrine glands, especially the adrenals and the thyroid.

IRRADIATION OF THE HYPOPHYSIS

Since the hypophysis elaborates a diabetogenic hormone and since its removal reduces the severity of experimental diabetes, it was thought that heavy doses of roentgen rays directed to the gland might produce similar results. A search of the literature revealed that Cushing (1932) had treated successfully with x-rays a patient with pituitary basophilism showing hyperglycemia (219 mgm. per cent) and glycosuria, and requiring sixty-five units of insulin daily. This patient became entirely sugar-free on an ordinary diet without insulin. The roentgen dosage used is not stated. Since undertaking the experimental work reported here, we have observed an article by Hutton (1933) implying that he has successfully treated diabetes by irradiation of the hypophysis. Neither the number of cases treated nor the dose employed is mentioned.

METHODS

In our studies seven dogs were rendered diabetic by complete removal of the pancreas. After recovery from the operation, which usually required about three weeks, the animals were kept on a basic diet of dog biscuits, sugar, and ground meat, to which was added liberal amounts of fresh or dried pancreas. On this stock diet two units of insulin per kilo of body weight was given twice daily at the time of feeding. Between periods of observation no special attempt was made to control the diabetic condition; the animals were seldom given sufficient insulin to render them sugar-free, yet they were never permitted to show a marked glycosuria or ketonuria. Prior to periods when the tolerance to glucose and insulin was determined, the animals were maintained in an approximately constant diabetic state. For each kilo of body weight the diet consisted of thirty grams of a stock meat preparation (Marco), five grams of sugar, and four grams of fresh pancreas or one gram of pancreatin (Parke-Davis). On this diet insulin was given twice daily in doses of two units per kilo of body weight. After the animals had been subjected to these standardized conditions for at least two days, food and insulin were withdrawn for a period of twenty hours and tolerance tests were made.

The glucose tolerance tests were carried out in the customary manner; following the taking of an initial blood sample, the animals were given 1.75 grams of glucose per kilo. of body weight, and subsequent blood samples were taken at 15, 45, 75, and 105 minutes. The glucose of the blood was determined by the method of Folin.

To test the animal's sensitivity to insulin, food and insulin were withheld for twenty hours during the period of the controlled diabetic state. Two to two and one-half units of insulin were then administered and the animal was observed over a period of four hours to see if convulsions occurred.

Following one or more control tolerance tests, the animals were given varying doses of x-ray. In order to render the animals immobile during the long treatment, they were anesthetized with nembutal, and tied to a support which held the head securely in any desired position. The rays were delivered to the

hypophyseal area through one dorsal and two lateral ports, each 5 cm. square. The physical constants employed were: 140 kilovolts (mechanical rectification) with 5 milliamperes, a focal skin distance of 50 cm., and 0.5 mm. copper and 1 mm. aluminum filtration. With these constants the Coolidge tube delivered 5.8 r. units per minute. The total dose given the hypophysis, as calculated by the Bachem tables, varied between 750 and 2025 r. for a single treatment. Four of the animals were given a second and heavier treatment about one month after the first. Subsequent glucose and insulin tolerance tests were carried out at weekly intervals following irradiation.

Histological studies were made of the pituitary and other irradiated areas of the brain, as well as the thyroid, adrenals, and gonads, to determine the effect of irradiation. The completeness of the removal of the pancreas was checked at autopsy.

RESULTS

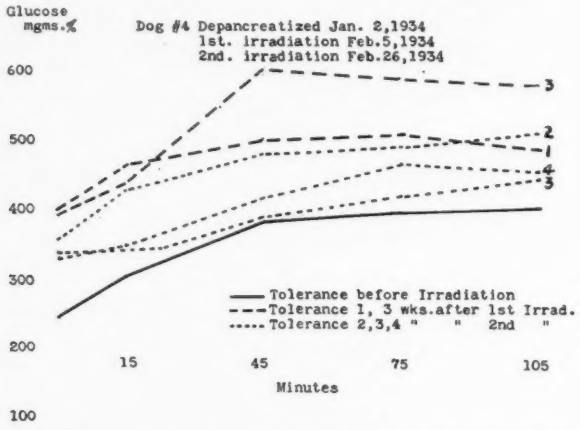
1. *Sugar Tolerance.* The data presented in Table I and in three graphs show a wide variation in the fasting blood sugar of most of the animals following irradiation. In two animals, Nos. 1 and 5, the sugar level was lower after treatment, suggesting that irradiation might have been

TABLE I

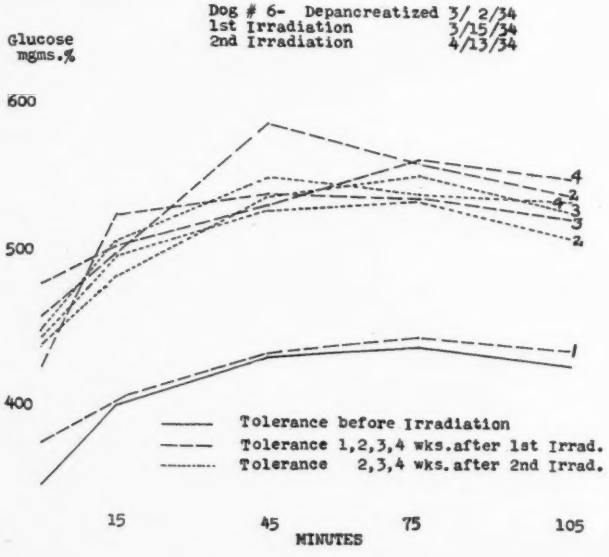
GLUCOSE TOLERANCE TESTS OF DEPANCREATIZED DOGS BEFORE AND AFTER IRRADIATION OF THE HYPOPHYSIS

	Dog No. 1	Dog No. 2	Dog No. 3	Dog No. 4	Dog No. 5	Dog No. 6	Dog No. 7
Depancreatized	10/8/33	1/1/34	11/22/33	1/2/34	12/12/33	3/2/34	3/24/34
GLUCOSE TOLERANCE BEFORE IRRADIATION							
Mgms. per cent glucose at 0, 15, 45, 75, 105 min.	498, 574, 606, 606, 613	370, 418, 459, 462, 465	186, 241, 305, 367, 357	246, 303, 385, 394, 400	207, 285, 377, 364, 316	346, 400, 432, 439, 439	319, 343, 412, 408, 396
First irradiation	1/2/34	1/24/34	1/13/34	2/5/34	2/12/34	3/15/34	4/6, 7, 9/34
Dose to skin per port	500r	500r	650r	700r	1000r	1350r	1350r
Dose to hypophysis	750r	900r	975r	945r	1500r	1820r	2025r
GLUCOSE TOLERANCE AFTER FIRST IRRADIATION							
1 week				400, 465, 500, 506, 485		374, 402, 436, 444, 437	381, 423, 491, 485, 470
2 weeks	377, 423, 432, 432, 430				170, 204, 269, 287, 296	471, 500, 585, 562, 541	
3 weeks	354, 392, 465, 449, 432	446, 458, 518, 540, 505	244, 280, 388, 439, 400	398, 444, 602, 588, 577	188, 266, 351, 245, 328	426, 526, 540, 537, 533	372, 414, 454, 452, 430
4 weeks						479, 506, 533, 563, 548	394, 437, 465, 468, 457
Second irradiation		2/16/34	2/9/34	2/26/34		4/13/34	
Dose to skin per port		810r	780r	900r		1300r	
Dose to hypophysis		1460r	1070r	1220r		1750r	
GLUCOSE TOLERANCE AFTER SECOND IRRADIATION							
2 weeks		555, 588, 658, 694, 641	265, 315, 408, 471, 436	361, 433, 480, 492, 508		444, 497, 533, 537, 520	
3 weeks		366, 390, 423, 430, 427		341, 343, 383, 426, 442		437, 485, 537, 551, 523	
4 weeks				333, 350, 422, 464, 458		444, 506, 552, 540, 536	

beneficial; the nutritive condition, however, of these animals became greatly impaired soon after irradiation, a fact probably accounting for the reduction in blood sugar. In all other animals the fasting sugar level was either unchanged or was actually higher than the initial pre-treatment level, as in the case of three animals, Nos. 2, 4 and 6, the blood sugar of which was

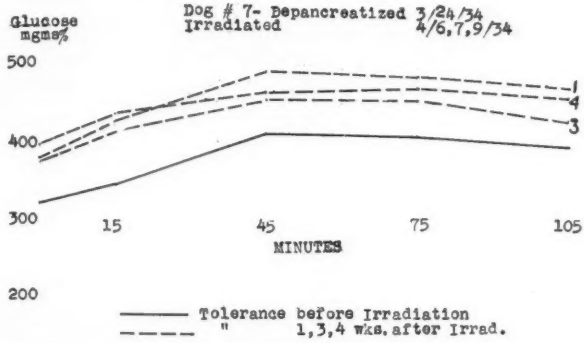


75 to 100 milligrams higher after irradiation. The course of the blood sugar after ingestion of glucose was the same after treatment as before. The highest value was attained in most animals after forty-five minutes; the concentration decreased thereafter at a rate of about 10 mgm. per cent for each succeeding half-hour period.



2. *Insulin Sensitivity.* Irradiation did not render the animals hypersensitive to insulin during fasting for convulsions never occurred in any of the animals given two units per kilo, and all animals but one (No. 5) tolerated two and one-half units per kilo.

3. *Glycosuria.* That glycosuria was not greatly reduced by irradiation was indicated by the fact that the two heaviest irradiated animals eliminated glucose in amounts nearly maximal for starving depancreatized animals. Thus, dogs Nos. 6 and 7 excreted 3.2 and 4.4 grams per kilo daily, respectively.



4. *Histological Changes Produced by Irradiation.* The histological studies made on six of the seven animals irradiated indicate that the hypophysis was not seriously damaged by the treatment (Table II). In dogs Nos. 4, 5, 6 and 7 the gland was slightly impaired, as indicated by edema and degeneration of the cells of the pars tuberalis. The nerve structures of the brain, however, showed more clearly the effects of the massive irradiation. Thus the cortex of all animals and the mid-brain of those animals receiving 1500 r. units or more of irradiation were definitely damaged.

DISCUSSION

It is difficult to reconcile the almost complete failure of this attempt to decrease the severity of symptoms of pancreatic diabetes with definitely proven facts regarding the hypophyseal-islet interrelationship. The amelioration of pancreatic diabetes by hypophysectomy has been demonstrated by too many competent workers to be questioned. It has, furthermore, been demonstrated that if only a small part of the anterior lobe be left intact, the diabetic symptoms persist (Daggs and Eaton). Our failure might, then, be explained by the fact that we have not produced adequate destruction of the anterior lobe in spite of severe damage rendered the surrounding nerve structures. This, however, seems inconsistent with reports (Hutton) that far less intense irradiation of the hypophysis in clinical cases of diabetes has produced marked improvement in patients. There are several respects, however, in which experimental pancreatic dia-

TABLE II

SUMMARY OF HISTOLOGICAL STUDIES

	Dog No. 2	Dog No. 3	Dog No. 4	Dog No. 5	Dog No. 6	Dog No. 7
Depancreatized	1/1/34	11/22/33	1/2/34	12/12/33	3/2/34	3/24/34
Killed	3/11/34	2/26/34	3/26/34	3/11/34	6/6/34	5/3/34
Duration of diabetic state	10 weeks	13 weeks	13 weeks	13 weeks	11 weeks	6 weeks
1st dose to hypophysis 2nd dose to hypophysis	900r 1460r	975r 1070r	945r 1220r	1500r	1350r 1820r	2025r .....
Autopsy after irradiation	6½ weeks	7½ weeks	8 weeks	4 weeks	9 weeks	4 weeks
Liver	Fatty degen. Necrosis	Fatty degen. Necrosis	Fatty degen. Necrosis	Fatty degen.	Fatty degen.	Fatty degen.
Ovary	Normal	.....	.....	Sclerotic	.....	Hyperplasia germ. epith. Many follicles
Testis	.....	Normal	Tubules degen.	.....	.....	.....
Adrenals	Slight congestion	Normal	Cortex and medulla moderate degen.	Cortex normal. Medulla slight degen.	.....	Cortex slight degen. Medulla normal
Thyroid	Normal	Normal	Atrophied	Normal	Atrophied	Proliferation of cells
Cortex	Congestion degeneration	Moderate degeneration	Marked degeneration	Marked degeneration	Marked degeneration	Marked degeneration
Mid-Brain	.....	Normal	Normal	Slight degeneration	Marked degeneration	Marked degeneration
Hypophysis— 1 pars nervosa	.....	Normal	Congestion Slight degeneration	Normal	Congestion. Marked degeneration	Normal
2 pars intermedia	.....	Normal	Slight degeneration	Normal	Marked degeneration	Normal
3 pars tuberalis	.....	Acidophil cells numerous	.....	Slight congestion and degen.	Marked congestion and degen.	Slight congestion and degen.

betes and diabetes mellitus are not strictly analogous. First, there is no complete pancreatic deficiency in diabetes mellitus; and second, there is probably no hyper-functioning anterior lobe, presumedly present in diabetes mellitus, in the depancreatized dog.

Since the physiological state of the depancreatized animal may not simulate the clinical condition, conclusions drawn here do not deny the reported beneficial effects of irradiation of the hypophysis in clinical diabetes. It is conceivable that in the disease, irradiation of the hypophysis may lead to improvement due to stimulation of the islet cells by some remote or obscure mechanism, rather than by depression of excessive diabetogenic activities of the anterior lobe. Or we may argue on the basis of the general claims of Fränkel that the supposedly hyper-functioning hypophysis in diabetes mellitus is more sensitive to roentgen rays than is the normal hypophysis of the experimentally produced diabetic dog.

In considering the effect of massive irradiation of the hypophyseal area, cognizance must be taken of the anatomical and functional relation-

ship existing between the hypophysis and the hypothalamus immediately above. In the latter are centers controlling various important metabolic processes, such as water exchange, heat regulation, and particularly the mobilization of glycogen from the liver. Massive irradiation must necessarily act on these nervous centers as well as on the hypophysis itself. Consequently any change which follows massive irradiation of the brain in this vicinity may not be due to the effect on the hypophysis alone but to the vegetative centers in the hypothalamus as well.

Whatever the explanation of the failure of these experiments may be, they sound a note of warning to workers contemplating the treatment of diabetes mellitus by irradiation of the hypophysis. Even massive doses of roentgen rays which one would not employ in treating patients seem to have very little or no inhibitory effect on the diabetogenic activity of the gland; a third of this dose to the skin, however, produces severe second degree burns. What immediate or delayed effects these heavy doses have on nerve structures and on other hormones elaborated by the hypophysis cannot be easily determined. Thus it seems to us that the irradiation treatment of diabetes mellitus requires a better rationale, which can proceed only from a more exhaustive clinical and experimental study of the details of the hypophysis-pancreas interrelationship.

SUMMARY

In five of seven diabetic (depancreatized) dogs the fasting blood sugar was not reduced after irradiation of the hypophyses by high dosage. In three animals the blood sugar was considerably higher after irradiation.

The response to ingested glucose was always typical of severe diabetes.

There was no increased sensitivity to insulin and the insulin dosage necessary to prevent glycosuria on a well-controlled diet could not be reduced.

No indication, then, was found that massive irradiation of the hypophysis with x-rays under the conditions stated, reduces the severity of the symptoms of experimental diabetes.

The authors wish to express their indebtedness to Dr. John Pilcher of the Department of Pathology for the histological studies reported in the paper.

REFERENCES

1. Anderson, K. W.: Minnesota Med. **12:** 484. 1929.
2. Anselmino, K. J., L. Herold and F. Hoffman: Klin. Wchnschr. **12:** 1245. 1933.
3. Anselmino, K. J. and F. Hoffman: Klin. Wchnschr. **10:** 2380. 1931.
4. Barnes, B. O. and J. F. Regan: Endocrinology, **17:** 522. 1933.
5. Borchardt, L.: Ztschr. f. klin. Med. **66:** 332. 1908.
6. Boyd, William: Pathology of internal diseases. Lea & Febiger, Philadelphia, 1931.
7. Corkill, A. B., H. P. Marks and W. E. White: J. Physiol. **80:** 193. 1933.
8. Cushing, H.: Johns Hopkins Hosp. Bull. **22:** 165. 1911.
9. Cushing, H.: Papers relating to the pituitary body, hypothalamus and parasympathetic nervous system. Charles C. Thomas, Springfield, 1932.
10. Daggs, R. G. and A. G. Eaton: Am. J. Physiol. **106:** 299. 1933.
11. Davidoff, L. M. and H. Cushing: Arch. Int. Med **39:** 751. 1927.
12. Epstein, A. A.: J. A. M. A. **85:** 29. 1925.
13. Fränkel, M.: Deutsche med. Wchnschr. **47:** 242. 1921.
14. Geiling, E. M. K. and S. W. Britton: Am. J. Physiol. **81:** 478. 1927.

15. Geiling, E. M. K., S. W. Britton and H. O. Calvery: J. Pharmacol. & Exper. Therap. **36:** 235. 1929.
16. Gordon, M. B.: Endocrinology, **14:** 411. 1930.
17. Houssay, B. A. and A. Biasotti: Endocrinology, **15:** 511. 1931.
18. Hutton, J. H.: Diabetes mellitus and essential hypertension. Illinois M. J. **64:** 539. 1933.
19. Joslin, E. P.: Treatment of diabetes mellitus. Lea & Febiger, Philadelphia, 1928. Fourth Edition.
20. Kutz, R. L., H. Selye, O. Denstedt, C. Bachman, D. L. Thomson and J. B. Collip: Amer. J. Physiol. **109:** 66. 1934.
21. Loeb, M.: Deutsches Arch. f. klin. Med. **34:** 443. 1883. Zentralbl. f. inn. Med. **19:** 893. 1898.
22. Macleod, J. J. R.: Physiology and biochemistry in modern medicine. C. V. Mosby Co., St. Lous, 1930.
23. Rowntree, L. G.: Proc. Staff. Meet. Mayo Clin. **2:** 217. 1927.
24. Soskin, S.: Unpublished.

Abstract Department

Medulloblastoma involving the kidney. Alyea, E. P., South. M. J. **26:** 753. 1933.

Eleven cases of medulloblastoma are tabulated with physical findings and autopsy and one is discussed in detail.—J. C. D.

Appearance of hyperglycemia in cats after stimulation of the peripheral end of the sympathetic nerve (Hyperglycémie chez le chat après stimulation du bout périphérique de nerfs sympathiques). Bacq, Z. M., Compt. rend. Soc. de biol. **112:** 701. 1933.

Cats were prepared by the method of Cannon and Bacq—adrenalectomy on the right side, denervation of the left adrenal and liver, bilateral thoracic sympathectomy and vagotomy, and section of the medulla. In five cats stimulation of the peripheral end of the cut sympathetic or of a motor nerve containing sympathetic fibers gave an increase in blood sugar. The rise is probably due to the action of sympathine, the hormone liberated in the blood stream by sympathetic stimulation.—J. C. D.

Adrenal cortex therapy in Graves' disease. Bram, I., Medical Rec. **140:** 67. 1934.

To 12 patients suffering from exophthalmic goiter was administered glycerine extract of adrenal cortex in doses of 9 to 12 ten-grain pills daily. Twelve similar patients were observed as controls, receiving the same general treatment but without the adrenal cortex. It was found that the inclusion of the cortex extract resulted in comparatively prompt relief from tachycardia, heightened basal metabolic rate, nervousness and fatigability. The relief occurred approximately eight weeks sooner than in the control patients. The average duration of the treatment was 10 weeks. The author believes that the adrenal cortex treatment is indicated in patients presenting the syndrome: little or no thyroid swelling, little or no exophthalmos, arterial hypertension, moderate loss in weight, rather stubborn tachycardia, with pulse rate from 130 to 160 per minute, and oxygen consumption rate varying between plus 40 and plus 70%. This group commonly responds rather tardily to the usual therapeutic remedy but promptly when glycerine extract of adrenal cortex is included in the regime. Comment is made on the paradox that in this type of patient cortex extract decreases the blood pressure as contrasted with its effect in some other subjects in which the pressure is increased.—R. G. H.

The relationship between status lymphaticus and the suprarenal glands. Campbell, E. H., Arch. Otolaryng. **15:** 517. 1932.

Marine defines status lymphaticus as follows: "It is a constitutional defect, usually congenital, though it may be acquired, dependent upon an inadequacy of the adrenal function, sex glands and autonomic nervous system, associated with lowered resistance or increased susceptibility to a great variety of non-specific, physical and chemical agents." Autonomically it is characterized by delayed involution or hyperplasia of the lymph glands and lymphoid tissue of the various organs, underdevelopment of the chromaffin, gonadal (suprarenal cortex, interstitial tissue of the ovaries and testes) and cardiovascular systems. Marine, Manley and Baumann believe that thymic and lymphoid hyperplasia of childhood are manifestations of functional underdevelopment of the suprarenal cortex and sex glands. Thymic enlargement follows extirpation of the adrenals. Since the suprarenals act to protect the body in emergencies such as flight, combat and infections, there seems to be a close relationship between thymus and adrenals. Some observers state that there is always a hypoplasia of the suprarenals in thymolymphaticus and that the enlarged thymus is only a symptom of status thymolymphaticus which in turn is due to deficiency of adrenal secretion.—M. B. G.

Gonad-stimulating action of pituitary from adrenalectomized rats (Acción excito sexual de la hipófisis de ratas suprarenoprivas). del Castillo, E. B., Rev. Soc. argent. de biol. **9**: 469. 1933.

Adult rats were adrenalectomized and 9 and 12 days later the pituitary was removed and implanted subcutaneously into 24-day-old female rats. Two glands were implanted on the first day, and 1 on the next. Litter mate controls received pituitary from normal rats. No difference was found in the genital tract (weight of ovary and uterus, opening of vagina) between both series. It was concluded that adrenalectomy did not modify the gonad-stimulating action of the pituitary gland.—J. T. L.

Thyroid-stimulating action of pituitary from adrenalectomized rats (Acción excito tiroidea de la hipófisis de ratas suprarrenoprivas). del Castillo, E. B., Rev. Soc. argent. de biol. **9**: 472. 1933.

The pituitary was removed, from rats 10 days after double adrenalectomy, and suspended in sterile saline. This extract was given intraperitoneally to young guinea pigs (1 gland per day on 2 successive days). Controls received pituitary from normal rats. The weight of the thyroids and their microscopic aspect was the same in both series. Adrenalectomy, therefore, does not modify the thyroid-stimulating action of the pituitary. The pituitaries from adrenalectomized rats were examined microscopically and found normal.—J. T. L.

Influence of cortical adrenal extract on endocrine glands (Influencia del extracto cortico-suprarrenal sobre las glándulas endócrinas). del Castillo, E. B., L. F. Leloir and A. Novelli, Rev. Soc. argent. de biol. **9**: 536. 1933.

Male rats (21) were injected with 0.5 cc. (15 grams fresh gland) of Swingle and Pfiffner's adrenal cortical extract daily for 20 days. The adrenals, thyroid, gonads and pituitary were weighed and examined histologically. There was no significant difference found between the glands of the treated animals and those of 18 controls. The adrenaline content of the adrenals and the thyroid and gonad-stimulating power of the pituitary, tested in guinea pigs and immature female rats, respectively, was not modified in treated rats as compared with the findings in normal controls. The extract was active since it protected adrenalectomized rats against morphin poisoning and increased muscle glycogen formation in adrenalectomized dogs.—J. T. L.

The ovarian cycle and the adrenal glands. Corey, E. L. and S. W. Britton, Am. J. Physiol. **107**: 207. 1934.

Adrenalectomy resulted in the complete suppression of the estrous cycle in 34 of 39 operated rats. Residual or accessory cortical tissues are possibly responsible for the persistence of estrus in a small percentage of adrenalectomized animals. The administration of cortico-adrenal extract to normal rats may (a) influence the ovarian cycle in the direction of increased activity; (b) produce no effect, or (c) result in an inhibition of the cycle. Cortico-adrenal extract restored the estrous cycle of adrenalectomized rats in all cases studied. This occurred either at once or after a lag period of a few days. In numerous experiments with theelin and antuitrin "S," it was not found possible to restore estrus when the materials were administered after adrenalectomy. When injections were begun before operation, however, the cycle was thereafter well maintained. No specific effect of cortico-adrenal extract on the ovarian cycle of spayed adult female rats was demonstrated.—Authors' Summary.

Subtotal bilateral suprarenalectomy for hypersuprarenalism (essential hypertension). DeCourcy, J. L., C. DeCourcy and O. Thuss, J. A. M. A. **102**: 1118. 1934. Abst., A. M. A.

In their studies the authors attempt to cut down the suprarenal hormone concentration of the blood. The treatment of essential hypertension, associated with little or no arteriosclerosis and no kidney destruction, has presented apparently insuperable difficulties to the internist. Little of permanent value has been accomplished in these cases, and they usually go on to a fatal termination. The authors have felt for some time that the picture is one of an endocrine dyscrasia and that the responsible glands are the suprarenals. Assuming that essential hypertension is due to excessive secretion of epinephrine by hyperplastic suprarenals, comparable to the excessive secretion of thyroxine in hyperplastic thyroids, they attempted to reduce the secretion by subtotal

suprarenalectomy in a number of cases. The suprarenal, because of the large network of nerve fibers connected with its capsule, has been called the brain of the vegetative nervous system. It seems logical, therefore, to find an analogy between the overactivity of the thyroid, due to sympathetic stimulation causing hyperthyroidism, and the overactivity of the suprarenals, due to sympathetic stimulation causing hypersuprarenalism. If this analogy is valid, partial suprarenalectomy should give results in hypertension comparable to those obtained from thyroidectomy for hyperthyroidism. In all their cases of hyperthyroidism in which an operation has been performed and which later exhibited recurrent symptoms of hyperthyroidism, the authors have been able to show an increase in the remaining hyperplastic tissues and have accordingly relieved their patient by again lessening the amount of the remaining tissue. In one case, partial thyroidectomy was performed five times, with eventual complete relief of symptoms and restoration of a normal basal metabolic rate. The reports in the literature support the authors' view that the most rational method of treatment of essential hypertension is the surgical excision of sufficien amounts of glandular tissue to relieve the excessive functioning of the gland itself. Their procedure for partial suprarenalectomy as performed by them consists of removal of about two-thirds of each suprarenal and is done in two stages. The portion removed includes both medulla and cortex and is taken from the part of the organ remote from the entrance of the blood vessels. The approach is made from the back through a kidney incision, the kidney being held down with a special retractor. The suprarenal must be recognized and stripped clean of all fat and overlying structures. The portion to be removed is then clamped and excised, the raw surface being covered with a continuous lock-stitch chromic suture. Spinal anesthesia is employed, and the blood pressure is watched closely throughout the entire operation. If the fall is more than anticipated, ephedrine is promptly given and intravenous saline solution with epinephrine administered in case of collapse. The partial suprarenalectomies that the authors have carried out have been so uniformly satisfactory that they feel that they are now relieving patients who previously were doomed to die.

Adrenal cortex and glycogen formation (Corteza suprarrenal y formación de glucógeno). Foglia, V. G., R. Fernández, L. F. Leloir and A. Novelli, Rev. Soc. argent. de biol. **9**: 522. 1933.

Adrenalectomized dogs were injected intravenously with 2 grams of glucose per kg. body weight 48 hours after operation. They did not show an increase in muscle glycogen; the liver glycogen increased somewhat but still remained below normal. Treatment with Swingle's adrenal cortical extract permitted normal glycogen storage by muscles and the amount of liver glycogen was greater than in the non-treated animals. Insulin had the same effect as the adrenal cortical extract. In pancreatectomized dogs treated with the adrenal cortical extract there was a slight increase of muscle glycogen after glucose injection in one animal, a definite increase in another and no variation in two. The rise in blood sugar after glucose injection was more pronounced and prolonged in adrenalectomized dogs, even when treated with extract, than in normal controls.—J. T. L.

Vagotonine and its adrenalinogenic effect in asphyxia (Vagotonine et effets adrenalinogènes de l'asphyxia). Franck, C. and L. Merklen.

Specificity of the action of vagotonine on the adrenalinogenic effects of splanchnic stimulation (Spécificité de l'action de la vagotonine sur les effets adrénalinogènes de l'excitation du splanchnique). Franck, C., L. Merklen and M. Vidacovitch.

Comparative oncographic studies of the action of vagotonine and of several substances of pancreatic origin (Etude oncographique comparée de l'action de la vagotonine et de diverses substances d'origine pancréatique). Hermann, H., L. Merklen, D. Santenois and M. Vidacovitch.

On the chemical individuality of vagotonine (Sur l'individualité chemique de la vagatonine). Fuchs, G. and L. Merklen, Compt. rend. Soc. de biol. **112**: 1353; 1355; 1358; 1362. 1933.

This series of articles on vagotonine, the method of its preparation, its specific hypotensive action, and its antagonistic action toward the effects of adrenine in the body apparently leave no doubt that it is an entity.—J. C. D.

Adrenal secretion in man; the reactions of the blood vessels of the human extremity, sensitized by sympathectomy, to adrenaline and to adrenal secretion resulting from insulin hypoglycemia. Freeman, N. E., R. H. Smithwick and J. C. White, Am. J. Physiol. **107**: 529. 1934.

The decrease in surface temperature following stimulation by cold, pain, or fear observed in patients with Raynaud's disease even after complete sympathectomy suggested the action of secreted adrenine. Continuous intravenous injections of adrenaline (1:250,000) into patients, following cervico-dorsal ganglionectomy, showed that the blood vessels of the sympathectomized extremity were more sensitive than those normally innervated. Injections of adrenaline were repeated 2, 6, 8, and 18 days after sympathectomy by paravertebral alcohol injection. The blood vessels were found to become sensitive between the 6th and 8th days. Injections of insulin sufficient to cause hypoglycemia consistently caused a fall in skin temperature on the sympathectomized side with no change or even a rise on the normal side. Since insulin hypoglycemia is known to cause adrenal secretion in animals, this response showed that the human adrenal medulla secretes sufficient adrenine to cause constriction of blood vessels sensitized by sympathectomy. Control experiments were performed on rabbits in which the adrenals could be inactivated. With the adrenals intact, insulin hypoglycemia produced a definite fall in the temperature of the denervated ear. After removal of one adrenal and denervation of the other, hypoglycemia to the point of convulsions resulted in no decrease in the surface temperature.—Authors' Summary.

Survival period of bilaterally adrenalectomized rats. Gaunt, R., Proc. Soc. Exper. Biol. & Med. **29**: 283. 1932.

A series of 156 rats from 5 different colonies were doubly adrenalectomized in order to check the discrepancies reported in regard to the survival period of this species. It was found that the life-span after operation was sufficiently varied to account for the previously divergent accounts. One strain (T-Colony) had an average life-span to date of 14.2 days, twice that of the other 4 colonies. Modification of the operative procedure, such as that of Pencharz, et al., and Freed, et al., did not affect susceptibility. However, young rats appeared to be somewhat more susceptible. Extended survival was attributed to the presence of minute amounts of cortical accessory tissue.
—C. tum Suden.

Weight of adrenals in dogs after hypophysectomy and lesion of the tuber cinereum (El peso de las suprarrenales de los perros hipofisoprivos o con lesion tuberiana). Houssay, B. A., A. Biasotti and P. Mazzocco, Rev. Soc. argent. de biol. **9**: 202. 1933.

Craneotomy was performed in 6 dogs. No significant variation in body weight, in size and functions of sexual glands, nor in weight of adrenals (as compared with that of 151 normal males) was seen. Extirpation of the posterior lobe, leaving the anterior lobe intact, did not produce atrophy of the adrenals in 8 dogs killed 60 to 235 days after operation. Galvano-cauterization of the tuber cinereum from the pituitary stalk to the mammillary bodies was performed in 11 dogs. The posterior pituitary lobe was found damaged in half of them, the anterior lobe was normal, the adrenals were not modified except in those examined the first week, when slight atrophy was found. In 16 dogs that died or were killed within the first month after hypophysectomy adrenals were atrophied in all except 2. In 23 dogs examined more than 1 month after hypophysectomy adrenal atrophy occurred in 75%. The average decrease in weight was 38% of normal, and the maximum decrease 77%. This atrophy was due to absence of the anterior lobe. Hypophysectomized puppies also had small adrenals but their weight was proportional to the body weight, which was also considerably below normal (dwarfs). In hypophysectomized thyroidectomized adult dogs (4) killed in excellent health there was considerable adrenal atrophy.—J. T. L.

Histological aspects of adrenals in hypophysectomized dogs (Histología de las suprarrenales de los perros hipofisoprivos). Houssay, B. A. and R. Sammartino, Rev. Soc. argent. de biol. **9**: 209. 1933.

Microscopical examination of the adrenals from hypophysectomized dogs was made. In 6 the posterior pituitary lobe was extirpated, in 11 the tuber

cinereum was cauterized, in 2 incomplete hypophysectomy was performed and 12 dogs were normal controls. Hypophysectomized dogs showed atrophy of the internal layers of the cortex which began soon after operation (4 days). When maximal only the outer (glomerular) layer had normal or greater width, its cells as those of the fascicular layer that remained free were filled with large drops of fat (Sudan) and cholesterol (Schultze's technic). Cells disappeared by a process of simple atrophy and areolar or vacuolar degeneration; there was no necrosis nor hemorrhage, nor active proliferation of connective tissue. In the other groups of animals no alterations were found except a slight and transitory atrophy after lesion of the tuber cinereum. The medulla was normal in all the series.—J. T. L.

The effect of extracts of the adrenal cortex on growth and the reproductive system of normal rats, with particular reference to intersexuality. Howard, Evelyn and A. Grollman, Am. J. Physiol. **107:** 480. 1934.

A moderate excess of the hormone of the adrenal cortex has no appreciable influence on the size of the gonads, the oestrous cycle, or the course of gestation in the rat. Contradictory previous results of others are attributed either to the presence of toxic impurities in the extracts used, or to natural variability among experimental animals. The growth of normal young rats is not appreciably influenced by a moderate excess of the hormone of the adrenal cortex. No causal relation of the adrenal cortex to intersexuality has been detected with the method employed. The frequent but not invariable coincidence of intersexuality or precocious sexual maturity with adrenal cortical tumor is probably a result of a common inducing factor.—Authors' Summary.

Adrenaline, adrenal glands and blood pressure (Adrenalin, Nebennieren und Blutdruck). Konschegg, T., Ztschr. f. d. ges. exper. Med. **81:** 559. 1932.

Determination of the adrenaline load of adrenal glands shows that the usual methods are inadequate to detect the whole amount present in the gland tissue. The adrenaline content of the gland extracts increases 50 to 200% if they are left standing for several days. The final high values are obtained almost immediately by acid extraction, especially if stronger acids (HCl) were used. The observations suggest that adrenaline is present in the adrenal glands in combination with some other substance, probably some lipoid from which it is separated by acid hydrolysis. In mixing adrenaline solutions of known strength with lipoid suspensions obtained by extraction from various organs, especially the adrenals, a gradual disappearance of the adrenaline was noted, but the original strength could be restored by adding acid. The lipoid-adrenaline compound does not give the chemical reactions of adrenaline, but its biological activity can be demonstrated by the use of the Krawkow-Pissemski rabbit ear preparation or by fresh strips from human arteries. Some of the blood plasma colloids, however, seem to inactivate the lipoid-adrenaline compound. Alcohol extracts of dried human blood serum or plasma contain a vaso-constrictor substance from which adrenaline splits off after treatment with acids. This could be shown chemically by the iron chloride reaction and biologically by means of rabbit intestinal preparations. Adrenaline could be liberated from these extracts also by dialysis. The vaso-constrictor power of blood sera or plasma of patients suffering from hypertension is considerably higher than in normal controls. Normal sera are still active at dilutions varying from 1:1500 to 1:6000, while sera from hypertensive patients are still vaso-constricting at dilutions varying from 1:10,000 to 1:30,000. The corresponding figures for blood plasma are lower and vary in normals from 1:400 to 1:1200 and from 1:1200 to 1:6000 in the hypertensive material. The concentration of the hypertensive substance in the blood or plasma extracts seemed to be proportionate to the blood pressure of the patient. The lowest values, considerably below the normal figures, were obtained in a case of Addison's disease.—M. A. Goldzieher.

Formation of an epinephrin-like substance in autolysing adrenal glands. Larrain, A. R., R. G. Roberts and M. M. Kunde, Proc. Soc. Exper. Biol. & Med. **31:** 534. 1934.

Autolysis of crushed, whole adrenal glands gives rise to a powerful pressor substance which may be obtained by dialysis. This substance gives all the

ordinary chemical tests for adrenaline, but differs from it in that it is not precipitated by concentrated ammonium hydroxide. It does not give a biuret reaction; is not destroyed by boiling; is adsorbed by animal charcoal and aluminum hydroxide and does not lose its physiological effect if exposed to air at room temperature for 30 days. Glacial acetic acid does not destroy its pressor effect. Inhibiting autolysis of the crushed adrenal glands by the addition of potassium cyanide prevents the formation of the pressor substance as determined by chemical and physiological tests.—Authors' Summary.

Action of extract of Trichocereus candicans and its active principles hordenin and salts of p-oxyphenylethyltrimethylammonium on adrenal secretion (Acción del extracto de Trichocereus candicans [Br. et Rose] y sus principios activos, hordenina o anhalina [p-oxifeniletildimetilamina] y sales de p-oxifeniletiltrimetilamonio sobre la secreción de adrenalina). Lewis, J. T. and F. Ludueña, Rev. Soc. argent. de biol. **9**: 352. 1933.

The extract Trichocereus candicans given intravenously in doses of 0.05 mg. per kg. stimulates adrenal secretion even after complete denervation of the gland. This was proved by numerous transfusion experiments (Tournade and Chabrol's technique) in which the left lumbo-adrenal vein of one dog was anastomosed with the jugular vein of another; in this last animal the effects of the adrenaline secreted were observed by registering the blood pressure and the volume of the denervated limb or spleen. Experiments following Anrep's technique for the demonstration of adrenal secretion (alternate interruption and freedom of the circulation of the gland with observation of the blood pressure and denervated limb volume) were also made with the same result. The iodine salt of the active principle, p-oxyphenylethyltrimethylammonium, extracted from the plant or prepared by synthesis, has quantitatively the same activity as the extract. The activity on adrenal secretion relative to the activity on the blood pressure is greater than that of nicotin. Hordenine sulphate (2-4 mg. per kg. intravenously) also stimulates adrenal secretion, even after complete denervation of the gland. Smaller doses (0.5 mg. per kg.) while still active on the blood pressure do not stimulate adrenal secretion.
—J. T. L.

Effect of yohimbin on the action of extract of Trichocereus candicans and its active principles, anhalin and candicin and on the excitability of the splanchnics (Efecto de la Yohimbina sobre la acción del extracto de Trichocereus candicans y sus principios activos, anahalina y candicina [p-oxifeniletiltrimetilamonio] y sobre la excitabilidad del esplácnico). Ludueña, F. P., Rev. Soc. argent. de biol. **9**: 449. 1933.

Yohimbin (Merck) in dosage of 1-2 mg. per kg. given intravenously diminished the rise in blood-pressure produced by extract of Trichocereus candicans, anhalin iodide and nicotin. Larger doses either suppressed all effect on blood pressure produced by these substances or else gave a biphasic effect, a small rise followed by a small drop in blood pressure. Yohimbin in small doses diminished and in large doses suppressed the rise in blood pressure and adrenal secretion produced by splanchnic stimulation. The adreno-secretory effect of nicotin was decreased by yohimbin. Adrenal secretion produced by extract of Trichocereus candicans, anhalin sulphate and candicin iodide was only slightly diminished by yohimbin.—J. T. L.

Effect of spartein on the action of the extract of Trichocereus candicans and its active principles, anhalin and candicin and on the excitability of the splanchnic (Efecto del sulfato de Esparteina sobre la acción del extracto de Trichocereus candicans y sus principios activos, anhalina y candicina [p-oxifeniletiltrimetilamonio] y sobre la excitabilidad del esplácnico). Ludueña, F. P., Rev. Soc. argent. de biol. **9**: 453. 1933.

In sufficient doses spartein suppressed or inverted the pressor effect of extract of Trichocereus candicans, candicin iodide, anhalin sulphate, nicotin and splanchnic stimulation. The adrenal secretion produced by these agents was also suppressed by spartein. The pressor effect of adrenaline was increased by spartein.—J. T. L.

Effect of cocain on the action of Trichocereus candicans and its active principles candicin and anhalin and on the excitability of the splanchnic (Efecto de la cocaiha sobre la acción del extracto de Trichocereus candicans y sus principios activos, anhalina y candicina, [p-oxifeniletiltrimetilamonio] y sobre la excitabilidad del esplácnico). Ludueña, F. P., Rev. Soc. argent. de biol. **9**: 457. 1933.

Small doses (2-3 mg. per kg.) of cocain chlorhidrate slightly increased the pressor effect of extract of Trichocereus candicans. This was due in part to sensitization to the adrenaline (and sympathin?) secreted. Adrenal secretion produced by splanchnic stimulation was slightly increased by cocain in small doses. Large doses diminished the pressor effect of splanchnic stimulation and extract of Trichocereus candicans or its active principles.—J. T. L.

Rôle of the adrenals in nicotin hyperglycemia (Papel de las suprarrenales en la hiperglycemia nicotínica). Leloir, L. F., Rev. Soc. argent. de biol. **9**: 474. 1933.

Nicotin (5 mg. per kg.) given intravenously to dogs produced a rapid and transitory increase in blood sugar. Simple laparotomy under ether anaesthesia reduced this hyperglycemia when the drug was injected 3 to 5 hours later. No increase and sometimes a decrease in blood sugar was seen when nicotin was injected 3 to 5 hours after double adrenalectomy. Section of the splanchnics with extirpation of the lumbar sympathetic slightly reduced the hyperglycemia when the drug was given 3 to 5 hours after operation. A few days later the hyperglycemic reaction was normal in these dogs. In another series one adrenal and the medulla from the other gland were extirpated. In this group nicotin either produced no increase or only a slight increase followed by a definite decrease in blood sugar. Experiments with adreno-jugular transfusion showed that nicotin given to the donor produced a discharge of adrenaline sufficient to produce hyperglycemia in the adrenalectomized receptor. The reduction of nicotin hyperglycemia after laparotomy is due to a diminished secretion of adrenaline since a given dose of adrenaline produced the same increase in blood sugar in normal and laparotomized dogs. This inhibition of adrenal secretion is not due to ether anaesthesia since nicotin hyperglycemia is not modified by administration of ether for 30 minues, 4 hours before giving nicotin. Ergotamin and atropin (1 mg. per kg.) do not significantly modify nicotin hyperglycemia.—J. T. L.

Studies on the function of the adrenal cortex; interrenal bodies and adrenal cortex (Studien über Funktion der Nebennierenrinde; Interrenalkörper und Nebennierenrinde). Reiss, M. and P. Herzog, Endokrinologie, **10**: 401. 1932.

Extracts were made from the interrenal bodies of certain selachians and their action tested on 13 rabbits and 1 cat. The extract lowered the blood cholesterol of rabbits as much as 55%. An amount of extract representing 0.5 to 2 gm. of interrenal body was equivalent to an extract of adrenal cortex representing 20 to 40 gm. of fresh gland. Following subcutaneous injection maximum effect was obtained in 2 to 3 hours. The cholesterol-lowering substance was most abundant in lipoid extracts, but was also present in smaller amount in watery HCl extracts. Two bilaterally adrenalectomized rabbits were kept alive for several days by administration of the extract and one adrenalectomized cat was kept alive for 28 days.—W. J. A.

Studies on the function of the adrenal cortex; the rôle of the adrenal cortical hormone in sulphur metabolism (Studien über die Funktion der Nebennierenrinde; die Rolle des Nebennierenrindenhormons im Schwefelstoffwechsel). Winter, K. A. and M. Reiss, Endokrinologie, **10**: 404. 1932.

Sulphur determinations were made according to the method of Lorant on the blood of singly and doubly adrenalectomized dogs and of dogs receiving cortical extract. An increase of 50-100% in the sulphur content was obtained in the doubly adrenalectomized group in from 24 to 54 hours after operation. A decrease in the sulphur level, maximum 25%, occurred in from 1 to 3 hours after the administration of 2.5 cc. to 5 cc. of cortical extract (prepared according to the method of Swingle and Pfiffner).—C. tum Suden.

The mechanism of melanoderma in the course of the pigmentary cirrhoses; rôle of endocrine glands (Du mécanisme de la mélanodermie au cours des cirrhoses pigmentaires. Rôle des glandes endocrines). Abel, E. and J. Girard, Rev. franç. d'endocrinol. **11**: 245: 1933.

Since the authors are dissatisfied with all the existing theories concerning the occurrence of melanoderma and siderosis in cirrhosis, they suggest an examination of the endocrine factors on the basis of the analogy between the melanoderma of cirrhosis and that of Addison's disease. The fact that numerous cases of melanoderma have been described in the course of hyperthyroidism, hypophyseal syndromes, and sexual dystrophies, suggests a common endocrine mechanism. The authors have selected 14 cases of pigmentary cirrhoses from the literature which show an affection of one or more endocrine glands. Among these, the gonads are thought to play the chief rôle in view of the frequency with which impotence, testicular atrophy, infantilism, and ovarian dysfunction have been observed. Signs of hypothyroidism such as falling of the hair and dryness of the skin are seen. Other signs such as asthenia and hypotension, although admittedly of a more common order, are thought, nevertheless, to coincide with certain suprarenal alterations. Histologically, the testicles show atrophy of the seminiferous tubules, increase in the interstitial cells, and alterations in spermatogenesis; the thyroid, atrophy with impoverishment in a colloid which is often basophilic; the pancreas, proliferation of the connective tissues and atrophy of the glandular parenchyma; the pituitary and suprarenals, less marked changes. In many cases there is an intracellular deposit of hemosiderin in the endocrine glands. Cases have been found also, in which endocrine lesions have been observed in ordinary cirrhosis without cutaneous pigmentation. The authors are uncertain whether the affection is a primary glandular dysfunction, whether the cirrhogenous poison alters the glands at the same time as the liver, or whether the hepatic toxemia is secondary to the cirrhosis. It does not appear to be due to impregnation with iron pigments, because the siderosis is often lacking. The classification of the pigmentary cirrhoses among the endocrine melanodermas is suggested.—C. C. Franseen.

Influence of pregnancy, increased sexual function in males, and of irradiated ergosterol on growth (Zur Kenntnis des Einflusses der Graviditäten und des Ergosterins auf das Wachstum). Agduhr, E., Upsala läkaref. förh. **38**: 1. 1932.

By accurate quantitative and histo-analytical methods applied to many litters of white mice, Agduhr finds that large doses of irradiated ergosterol retard growth—particularly of adipose tissue. Pregnancy stimulates body growth, which is more pronounced if ergosterol is administered. This extra increase in body weight is no index of increased individual organ growth. The responses of the organs to ergosterol in sexually inactive animals also varies with different organs. Increased sexual function in males on a basal diet supplemented with ergosterol retarded growth of the skeleton. Augmented sexual function also seemed to retard the growth of the hypophysis of males. Pregnancy and lactation decreases the inorganic substances of the skeleton even when large quantities of ergosterol are given. Many of the growth changes studied (of which only some are here mentioned) are believed to be determined more or less by many of the ductless glands, especially the gonads, hypophysis, thyroid and parathyroids. The author then proceeds to examine the latter three endocrine structures for changes in their size and histology. It was found that ergosterol increases the hypophysis of females but not significantly in males. In fact, the anterior lobe of the male may be slightly diminished. In the anterior lobe of the female ergosterol increases the number of glandular cells by 40%, and the mass by 70%. The acidophils show the greatest increase in number. The number of cells in pars intermedia is also increased 40%. With an increase in the number of times an animal becomes pregnant (without ergosterol) the anterior and posterior lobes increase. In the anterior lobe the number of cells are increased by 100%; the total volume of the cells by 250% and the total secreting surface by about 190%. The greatest absolute increase in number of cells in multipara is in the chromophobes (indifferent cells) but the greatest relative increase (290%) is shown by the acidophils. An increased number of pregnancies in cooperation with ergosterol augments the increase in weight of the whole hypophysis (due to enlargement of the anterior lobe) to a greater extent than either factor acting separately; the acidophils being increased by about 830%. The thyroid gland

is retarded by irradiated ergosterol in males but only slightly, if at all, in females. Increased number of pregnancies augments growth of epithelial elements and colloid of the thyroid gland. If in addition ergosterol is given, the epithelium is increased but not the colloid. Increased sexual function in the male both with and without ergosterol, stimulated growth of the epithelium, but not of the colloid in the thyroid. The parathyroids are inhibited by irradiated ergosterol; more so in males than in females. Increased number of pregnancies accelerate markedly the growth of the parathyroids in animals on basal diet (without ergosterol). The effect of increased sexual function on the parathyroids of males was not sufficient to be statistically significant.—A. T. R.

The use of thyroid and suprarenal in the diathesis of infancy. Barbour, O., Illinois M. J. **61:** 345. 1932.

During the past two years the author has found that gastro-intestinal symptoms such as vomiting, intestinal colic, flatus and constipation of diathetic children are usually relieved by the administration of thyroid. In each case with respiratory and circulatory symptoms such as laryngeal stridor, dyspnea, breath-holding, coughing and cyanosis, almost immediate relief followed the administration of suprarenal gland extract by mouth. Those cases showing both gastro-intestinal and respiratory symptoms respond favorably to combined thyroid and suprarenal therapy. Suprarenal therapy seems affective in eczematous cutaneous lesions that are present in some of these children.
—M. B. G.

Endocrine glands in recovery of muscle glycogen after fatigue (Papel de diversas glándulas endócrinas en la recomposición del glucógeno muscular después de la fatiga). Dambrosi, R. G., Rev. Soc. argent. de biol. **9:** 563. 1933.

Extirpation of the thyroid, parathyroid or gonads did not modify the capacity of muscle to restore glycogen after fatigue in dogs. Ingestion of large doses of thyroid diminished this capacity.—J. T. L.

Parathyroids in pituitary and pancreatic insufficiency (Las paratiroides en las insuficiencias hipofisaria y pancreatica). Houssay, B. A. and R. Sammartino, Rev. Soc. argent. de biol. **9:** 236. 1933.

Hypophysectomy produced atrophy and degenerative lesions in 66% of the dogs. The same alterations were found in 41% of the animals with cauterized tuber cinereum and 10% of the controls. They were frequently seen after pancreatectomy. Treatment of short duration with alkaline anterior pituitary extract did not produce regression of the lesions.—J. T. L.

The adrenals, liver, pancreas and vegetative nervous system in posterior pituitary extract hyperglycemia (Papel de las suprarrenales, higado, pancreas y sistema nervioso vegetativo en la hiperglycemia por extracto retro-pituitario). Houssay, B. A. and E. di Benedetto, Rev. Soc. argent. de biol. **9:** 310. 1933.

Intravenous infusion extract of posterior pituitary standard powder (2.5 mg. per kg.) produced a maximum increase in blood sugar of 26 mg. on an average. This increase did not occur in dogs after acute hepatectomy. Section of both splanchnics decreased the hyperglycemia slightly and section of both vagi slightly increased the hyperglycemia produced by the extract. It cannot be affirmed that the extract increased insulin secretion since there was no secondary hypoglycemia following hyperglycemia and pancreatectomy (1 to 2 hours before) did not enhance the hyperglycemic activity of the extract. The rise in blood sugar due to lack of insulin secretion occurred later. In dogs recently adrenalectomized the dogs were also pancreatectomized. Therefore, this drop was not due to an increase in insulin secretion. The injection of posterior pituitary extract did not increase adrenal secretion, as determined in experiments with adrenal-jugular transfusion (Tournade and Chabrol's technique). The continuous injection of adrenaline into adrenalectomized dogs, in doses corresponding to the amount normally secreted or very slightly higher, simultaneously with injection of posterior pituitary extract, was followed by hyperglycemia. The same dose of adrenaline alone in adrenalectomized dogs produced a slightly higher blood sugar level than that seen in the controls. It is deduced that the posterior pituitary hyperglycemia is due to a humoral action on the liver, requiring the secretion of adrenaline.—J. T. L.

Endocrine dyscrasias and mental disorders; preliminary report. Hutton, J. H., R. Brandon, C. F. Read and J. T. Nerancy, Illinois M. J. **64:** 242. 1933. Abst., J. Nerv. & Ment. Dis. **79:** 450.

These observers attempted to determine whether there was a relationship between endocrine dyscrasias and mental disorders and also whether the correction of the endocrinopathy would be valuable in the treatment of mental disorders. The report is very uncritical. The pituitary products used were a commercial soluble extract from the anterior lobe of the pituitary (antuitrin) and posterior lobe extracts. They were administered hypodermically three times a week. The dose of posterior lobe extracts was kept below that which produced unpleasant abdominal cramps. Ovarian medication consisted of the residue or of one of the standardized preparations, more often the residue, and one ampule was given subcutaneously three times a week. If a standardized preparation was used, it was given on alternate days beginning a week before the expected menstrual period. Thyroid has been given by mouth. Of 172 patients recognized as having some endocrine defect, 26.7% are now paroled or discharged, whereas the discharged and paroled patients from all the state institutions during the year ended June, 1931, were only 17.3%.

Experimental researches concerning the relation of the internal secretory glands to wound healing (Experimental Untersuchungen über de Beziehungen der innersekretorischen Drüsen zur Wundheilung). Lauber, H. J., Beitr. z. klin. Chir. **157:** 244. 1933.

An attempt was made to determine the influence of various hormones on wound healing. The research was carried out on white mice on the backs of which the skin was excised for one square centimeter. The normal healing time was established, by observing 75 untreated mice, as 22-25 days for complete epithelialization. The mice treated with the various hormones were divided into three groups. In the first, the animals were treated daily with therapeutic doses of the hormones for 14 days before making the wound. In the second, the animals were treated beginning at the time of making the wound. In the third group, the hormone was injected directly at the edge of the wound. The results with the various hormones in the three series ran parallel regardless of the method of administration. There was a marked acceleration of healing with Elitryran, Prolan, and Padutin and in the case of the females with Unden. There was a moderate acceleration of healing with Hypophysin and Adrenalin, while Erugon accelerated the healing moderately with male mice and to a less degree with female mice. Insulin had no influence upon wound healing and parathyroid extract delayed healing somewhat.
—R. W. Te Linde.

Influence of the endocrine organs on the teeth and jaws of children (Der Einfluss der endokrinen Drüsen an den Zähnen und Kiefern der Kinder und Jugendlichen). Trivus-Kaz, F. A., Ztschr. f. Konstitutionslehre, **17:** 1. 1932.

The author presents numerous cases with hypo and hyperfunctional as well as distrophic conditions of the ductless gland with accompanying abnormalities of the teeth and jaw. He concludes that the pineal gland, thymus, thyroid and parathyroids are very intimately connected with the development of the crown of the teeth. The hypophysis and sometimes also the thymus and thyroid influence not only the crown of the tooth but also the period of eruption and the position of the teeth in the jaw. The sex glands and suprarenals also play a part in above processes through interrelations with the abovementioned glands. In spite of the great diversity of form impressed upon the teeth and jaw by the ductless gland, the particular ones responsible for a given complex can usually be identified.—A. T. R.

Effect of folliculin on compensatory hypertrophy of ovary (Acción de la foliculina sobre la hipertrofia compensadora del ovario). Bialet-Laprida, Z., Rev. Soc. argent. de biol. **9:** 136. 1933.

One ovary and one-half of the other ovary of 15 rats weighing 95-120 gm. were extirpated. Ten animals were injected daily with 2 R.U. of folliculin for 34 days; 5 were left as controls. These latter had numerous mature follicles and corpora lutea in the remaining half ovary, which weighed 39 mg. (1 mg. ovary for 3.7 gm. body weight). In the treated animals the half ovary

weighed 13 mg. (1 mg. ovary for 9.3 gm. body weight). Only 3 animals showed a few corpora lutea, the follicles were small or medium sized, and the general aspect was that of an infantile gland.—J. T. L.

Action of folliculin on hypophysis (Acción de la foliculina sobre la hipófisis). Bialet-Laprida, Z., Rev. Soc. argent. de biol. **9**: 230. 1933.

Female rats weighing 115-125 grams were divided into groups of 12. One group was castrated, permanent dioestrus established and controlled by vaginal smears; another group was castrated and received 2 units of folliculin daily from 10 days before to 30 and 51 days after operation; the third group received 2 units folliculin daily for 30 and 52 days; the fourth group was the normal controls. The pituitaries from these rats were implanted subcutaneously, 2 on the first day and 1 on the second day into immature rats (35-45 grams). One hundred hours after the first implantation their ovaries were examined. Implantation of glands from the first group induced puberty (oestrus smear) in all cases. The ovaries weighed 29 mg. in the rat receiving pituitary from rats castrated 30 days previously, 33 and 44 mg. from rats castrated 39 days, and 98 mg. from rats castrated 50 days before. There were numerous mature follicles and corpora lutea. Implantation of glands from group two did not induce puberty. The genital tract was infantile and the ovarian weight was 19-21 mg. Pituitaries from non-castrated rats, treated and not treated with folliculin (groups three and four) had only a slight sexual effect. The ovaries were always found to be infantile. It is concluded that folliculin inhibits the production of the gonad-stimulating hormone of the pituitary.—J. T. L.

Effect of folliculin on ovarian grafts (Accion de la foliculina sobre el injerto ovarico). Bialet-Laprida, Z., Rev. Soc. argent. de biol. **9**: 253. 1933.

Rats (28) weighing 120-205 grams were castrated. Twenty-six to 35 days later 2 rat units of folliculin was injected daily. Ten days later an ovary was grafted into the kidney and the folliculin treatment continued for 30 days, at which time the grafts were examined microscopically. In only 5 animals small and medium sized follicles were found; in 23 no follicles nor corpora lutea were seen, only cysts and sclerosed tissue. Another series of 20 rats (135-245 grams) were spayed. Thirty days later the ovary was grafted and folliculin injections (2 rat units) were commenced and were continued for 30 days. At the end of this time small and medium sized follicles were found in 5; in 5 there were mature follicles and corpora lutea; in 10 no specific structures were seen. A third series of 13 rats (134-245 grams) were spayed, grafted and folliculin treatment (2 rat units daily) begun simultaneously; 30 days later mature follicles and corpora lutea were seen in 5; small and medium sized follicles were present in 5 and in 3 there were no specific structures. On increasing the dosage to 5 rat units daily in the same conditions as series three, no follicles were seen in 3 and only small or medium sized ones in 9; none had mature follicles nor corpora lutea. A control series of 41 rats was spayed, an ovarian graft was made 30 days later and folliculin was not injected; prolonged oestrus was seen and after 30 days there were mature follicles and corpora lutea in 12, small and medium follicles in 6 and no specific structures in 22. It is concluded that folliculin treatment before grafting is unfavorable to the grafted tissue, producing regression of the follicles. Folliculin when started at time of grafting favors the taking of the grafts but in large doses produces regression of follicles. It is believed that folliculin may inhibit the anterior pituitary secretion.—J. T. L.

Exact testing of the specific hormone of the corpus luteum (Supplementary note on the luteinizing hormone in the infant rabbit) (Zur exakten Testierung des spezifischen Hormons des Corpus luteum [Ergänzends Bemerkungen zum Luteohormontest am infantilen Kaninchen]). Clauberg, C., Klin. Wchnschr. **10**: 1949. 1931.

The relative merits of the proliferative test and the development of pituitrin insensitivity are considered. It is indicated that the uterus may become insensitive to pituitrin by withdrawing, or suddenly diminishing the dose of oestrin in an immature or castrate animal. Furthermore, if a preparation of corpus luteum be contaminated with considerable oestrin, the injection of such extract into an animal may produce a condition in which the uterus will react to pituitrin in vitro. For such reasons the author considers the histological

condition of the mucosa more nearly specific than the motor reactions and he also gives reasons for preferring the immature rabbit uterus, to that of the mature rabbit, or that of the mouse or rat. Significant contamination with oestrin can be detected by an unusual increase in the number of nuclei in the uterine muscle. A definition of the hormone unit is given together with explicit directions for assay.—M. H. Friedman.

Some factors influencing nitrogen economy during pregnancy. Coons, Callie M. and Gladys B. Marshal, J. Nutrition, **7**: 67. 1934.

Twenty-three 4-day balance experiments during pregnancy and one 4 weeks prior to pregnancy on six women are presented in this report. The results show relatively lower nitrogen retentions than those of previous studies, except when the diets were increased with additional protein or supplemented with accessory factors, particularly the vitamin B complex. The average intake, including supplemented diets, was 11.58 gm. of nitrogen daily with a storage of 1.97 gm. Tendencies to greater absorption rates as shown by higher coefficients of digestibility and by lower fecal residues were observed in this series and are discussed in relation to the metabolism of other elements. A knowledge of the nutritional history of the mother, both in the pregravid period and through all of gravidity is essential to the proper interpretation of nitrogen retentions during pregnancy.—Authors' Abst.

The length of the human menstrual cycle. Fluhmann, C. F., Am. J. Obst. & Gynec. **27**: 73. 1934.

An analysis of 747 accurately recorded menstrual cycles of 76 healthy young California women has shown a marked variability in their length. The range extended from eleven to over one hundred days, although the vast majority was between 18 and 42 days. The mean was 30.4 days, and the standard deviation ± 11.53. The individual cases were divided into two main groups. The first included 28, or one-third, of the subjects, in whom there was a certain degree of regularity in the lengths of successive menstrual cycles. Of these, 9 had cycles of 26 to 30 days' duration, while in 6 they tended to be shorter, and in 13 they tended to be longer than 26 to 30 days. The second group comprised 48, or two-thirds, of the cases and was characterized by a marked irregularity in the lengths of successive cycles. In 4 instances all the cycles were shorter than 30 days, in 16 they were longer than 26 days, while in 28 they were very irregular, some being shorter than 26 and others longer than 30 days. The average duration of the hemorrhage in 823 menstrual periods was 4.6 days. It varied from 3 to 7 days in the majority of instances, and no relation between the length of the menstrual cycle and the duration of the flow could be demonstrated.—Author's Summary.

Conditions of hypertrophy of the seminal vesicles in rats. Freud, J., Biochem. J. **27**: 1438. 1933. Abst., Chem. Absts. **28**: 1394.

Using young, castrated rats, the author finds that an optimum growth of seminal vesicles (S.V.) is not obtained with the male hormone (M.) alone. The effect of M. on smooth muscle is only moderate, its main effect being on the columnar epithelium. Estrin and trihydroxyestrin act on smooth muscle tissue, while they do not affect the columnar epithelium or induce degeneration. The combined effects of follicular and male hormone upon S.V. is a mutual reënforcement of each others' actions ("pacemaking"). Simultaneous experiments on capons and on infantile male rats show loss of activity of M. after purification as regards rats, while the activity is retained as regards capons. Estrin in crystalline form up to 1000 mouse units a day has no influence on comb growth; the seminal vesicles, however, are distinctly influenced.

Artificially induced ovulation in the cat (felis domestica). Greulich, W., Anat. Rec. **58**: 217. 1934.

Ovulation was induced in nine of 12 domestic cats by stimulating the distal portion of the genital tract with a glass rod. The length of the interval between stimulation and ovulation was found to be no more than 25 hours. Evidence is advanced to show that ovulation does not invariably follow normal coitus in the cat, even if the female is clearly in heat when the mating occurs. This is interpreted as favoring the view that the capacity for ovulation is not

always associated with an oestrous cycle in this form. The results of this investigation definitely eliminate all constituents of the seminal fluid as necessary causative factors in ovulation.—Author's Abst.

The clinical use of a preparation of oestrin in the treatment of functional secondary amenorrhoea; a record of 16 cases. Haultain, W. F. T., Tr. Edinburgh Obst. Soc., Edinburgh M. J. **40:** 94. 1933.

The theelin (progynon) was given by mouth in 150 mouse units, except in a few instances in which injections were used. Case histories are included. Eleven patients were cured, 3 received doubtful benefit, and 2 failed to respond.
—J. C. D.

Symptomatology and microscopic anatomy of granulosa cell tumors of ovary (Weiterer Beitrag zur Klinik und mikroskopischen Anatomie der Granulosozelltumoren des Eierstockes). Klafter, E., Arch. f. Gynak. **150:** 643. 1932.

Granulosa cell tumors develop from misplaced granulosa tissue. Ripe follicles are never the cause of granulosa cell tumors, while inborn misplacements of the granulosa cells may be. The granulosa cell tumors often develop many years after menopause at a time when normally no follicles are present. All tumors in which the cell prototype shows elements similar to the granulosa cells should be called granulosa cell tumors. Because of the predominance of certain morphologic structures, which are due to further developmental grades of the elements of the granulosa cells in the tumors, they can be differentiated as granulosa cellularis follicularis, folliculoids, cylindromatosus, plexiformis, adenomatous, luteiniformis, gyriformis and diffusus. There is mostly a combination of several kinds in the same tumor. In the diagnosis and recognition of the granulosa cell tumors the kind of cell elements from which the tumor is constructed is of significance. Meyer gave a good classification of these tumors. He also was the first who recognized the hormonal effect these tumors have on the genital apparatus, especially the uterus. There is an accentuation in the female secondary sex characteristics. The author has observed an increase in the basal metabolism up to 24%, which has returned to normal after the operation. The finding of structures similar to corpus luteum or corpus albicans explains the prolonged amenorrhoea. The granulosa cell tumors represent 4.04% of the ovarian tumors. They occur in any age, but are more common in the age of the full sexual development and in the climacterium. The most common age is from 30 to 60. The prognosis of the granulosa cell tumors is relatively good. Metastases occur mostly after the extirpation of the primary tumor and not simultaneously. It is possible that the same factors, which cause the ripe follicle and corpus luteum to inhibit the riping of other follicles may play a part in the suppression of the metastases. The author has seen 10 cases of granulosa cell tumors and reports some of them in detail. One patient presented a combination of adeno-carcinoma of the stomach with metastases and a granulosa cell tumor. The patient was operated upon because of the tumor, and the carcinoma of the stomach was accidentally discovered during the operation. At the autopsy the metastases were found to be due to the carcinoma of the stomach. The granulosa cell tumor was benign. The second case illustrates the benignity of the granulosa cell tumors. In spite of the fact that in this patient the tumor filled out almost the entire abdomen and one of its cysts ruptured during the operation, no metastases developed. A third patient developed a granulosa cell tumor for the second time eleven years after the extirpation of the first tumor. Whether the tumor was a recidive, metastatic or new, the author is unable to say. With the occurrence of the tumor for the second time, amenorrhoea alternated with the bleeding. From over 100 cases collected from the literature 20 had to be discarded because of insufficient data. From the 80 cases six (7.5%) were described as inoperable, five (6.2%) had the tumors on both ovaries. In four cases (5%) recidives or metastases occurred. The operation should be carried out by the abdominal way in order to avoid cutting into the tumor and eliminate the danger of implantation of the tumor in the peritoneal cavity. The prognosis of the recidive operation is also good. Also x-ray treatment is of value. The recidive and metastases occur after years. The granulosa cell tumors are sensitive to x-ray treatment similar to the granulosa cells of the ovaries. Although in many cases of the granulosa cell tumors formations similar to corpus luteum are found, it has not been proven that the lutein cell-like formations are identical with the cells of the true corpus luteum or that their function is the same. These corpus luteum-like structures are of value, however, in

the differential diagnosis. The follicle riping process in the normal ovary is often inhibited by the hormone of the granulosa cell tumor of the other ovary, but the effect is not lasting. It disappears with the widespread degeneration and necrosis within the tumor or with the extirpation of the same. There is usually present an abnormal hair growth of the masculine type, but this is apparently not due to the tumor. It is probably due to the underfunction of the ovaries. Nine microphotographs and one chart are given to illustrate the cases.—A. Rudy.

Note on the sensitiveness of uterine muscle from rabbits to the hormone of the posterior lobe of the hypophysis. Variations in the course of the ovarian cycle and in the course of pregnancy (Sur la sensibilité du muscle utérin a l'hormone post-hypophysaire chez la lapine. Ses variations au cours du cycle ovarien et au cours de la grossesse). Klein, M. and L. Klein, Compt. rend. Soc. de biol. **112:** 821. 1933.

In rabbits the uterine muscle, when suspended in Tyrode's solution at 38°, fails to respond to pituitrin in the presence of corpus luteum extract. Its responses are not exactly parallel to those of the uterine mucous membrane. Like it, however, it is an absolute test for activity of the corpus luteum.
—J. C. D.

Theca cell tumors of the ovary. Melnick, P. J. and A. E. Kanter, Am. J. Obst. & Gynec. **27:** 51. 1934.

Certain ovarian tumors exert hormonal effects and of the feminizing type there are two, the granulosa cell tumors and the theca cell tumors. Theca cell tumors have only recently been recognized. Two such cases are reported. They produced hyperplasia of the myometrium and endometrium, and post-climacteric bleeding. In one case the bleeding was periodic, resembling the normal menstrual cycle. The tumors are composed of cells which have the histologic characteristics of theca interna cells. Apparently these tumors secreted theelin. The conclusion that the theca cells secrete the estrogenic hormone is supported by much experimental and deductive evidence from the literature.—Authors' Summary.

The existence in the interstitial tissue of the mouse ovary of cells with physiological properties similar to those of the interstitial cells of the testis (Existence, dans le tissue interstitiel de l'ovaire de souris, de cellules douées de propriétés physiologiques analogues a celles des cellules interstitielles du testicule). Moricard, R., Compt. rend. Soc. de biol. **112:** 1045. 1933.

Injection of urine from pregnant women, implantation of hypophysis or of placenta in immature mice, produces a hyperplasia of the interstitial tissue. Certain hypophyseal extracts produce follicular maturation without ovulation or oestrous. In such cases the interstitial cells do not increase. After x-ray the ova die, the interstitial tissue hypertrophies, and the animal goes into continuous oestrous. This is evidence that the interstitial tissue of the ovary includes a "gland of oestrous" and plays an essential part in maintaining the cycle.—J. C. D.

The gonads in the development and larval metamorphosis of the tadpole; the action of the serum of castrated male animals (Le gonadi nello sviluppo e nella metamorfosi larvale; l'azione del siero di sangue di animali maschili castrate). Parenti, G. C., Sperimentale, Arch. di biol. **87:** 49. 1933.

Tadpoles of Bufo vulgaris and Rana esculenta, fed with serum of castrated male animals, showed a tendency to precocious development. The author supposes that, on account of the hormonic disturbance due to castration, an excessive quantity of pituitary and thyroid hormones is present, which causes precocious metamorphosis.—A. E. Meyer.

The abnormal excretion of theelin and prolan in patients suffering from migraine; preliminary report. Riley, H. A., R. M. Brickner and R. Kurzrok, Bull. Neurol. Inst. New York, **3:** 53. 1933.

Hormonal studies were made daily, over a prolonged period, on the urine of 13 patients (11 females and 2 males) suffering from migraine. The period of observation, in each instance, included a typical attack. In the female

patients a quantitative estimation of the female sex hormone and the identification of prolan were made. In the male patients prolan identification only was included. No distinction was made between prolan A and prolan B. Theelin was detected occasionally in the urine of all but one of the menstruating women and in this one the menopause was imminent. In no instance did the quantity reach the 10-20 rat units which is considered the normal amount for women within the menstrual age. In one woman who had passed a natural menopause and in another who had had induced menopause no theelin was detected. No demonstrable relationship could be determined between variations in excretion of theelin and the occurrence of headache. Prolan was excreted in all the cases. In the two women past the menopause and the one approaching that period prolan was present almost daily in large quantities; in the remaining 11 cases the hormone was present intermittently and in varying amounts. In the entire group of patients 29 individual headaches occurred. In 20 of the 29 the headache was preceded or accompanied by the presence of prolan in the urine. In two of the patients presenting status migrainicus, the excretion of prolan was practically uninterrupted. There was, therefore, a continuous relationship between the appearance of prolan and the occurrence of headache. In 9 of the 29 headaches no prolan-headache relationship was demonstrated. In 7 of the 9, however, the determination of an exact relationship between the appearance of prolan and the occurrence of headache was rendered impossible by the absence of urine specimens. In the remaining 2 the headache occurred without the antecedent appearance of prolan. Occasionally prolan appeared without the subsequent development of headache. No definite difference could be determined in the relationship between menstruation, headache and prolan excretion and the headache-prolan relationship occurring at other times. Nine of the female patients were injected with 2 cc. follutein and 7 developed an attack of migraine within 4 to 12 hours. Follutein was administered twice to one of the male patients with an interval of 6 days between the injections. A mild headache occurred three days after the first injection but there was no headache subsequent to the second injection.
—R. G. H.

Diabetes and pregnancy. Ronsheim, J., Am. J. Obst. & Gynec. **25**: 710. 1933.

The author reports the outcome of three pregnancies in young diabetic mothers. The first was 23 years old and had had diabetes for three years. At her second pregnancy she was delivered of a still-born baby weighing 5 lbs. 6 ozs. The mother was well and the diabetes was controlled on 15-15 units of insulin. The second case was that of a diabetic mother 29 years old. Her first child was still-born; her second pregnancy ended in miscarriage at 5 months; her third pregnancy in spontaneous delivery of a macerated fetus weighing 8 lbs. 6 oz. The diabetes is now controlled on diet alone. The third case was that of a diabetic mother 31 years old. At her first pregnancy she was delivered of a normal baby which died on the 18th day in deep jaundice. Her second pregnancy resulted in a premature birth. The child is living but is mentally deficient. The third pregnancy ended 10 weeks before term. A 3-lb. baby girl was born spontaneously and died in 24 hours. The mother shows no glycosuria now, has a normal blood sugar, but a glucose tolerance curve is diabetic in type. The author points out that in the Jewish hospital at Brooklyn in a total of 121 pregnancies in diabetic mothers there were 74 live babies and the fetal mortality was 38.8%. Latent diabetes, he says, is a greater menace to the fetus than frank diabetes. Uncontrolled diabetes will almost invariably result either in miscarriage or premature birth or the death of the fetus in utero. The Caesarean section is the method of choice in delivery. Ether and chloroform should be avoided. In cases of miscarriage or still-births the glucose tolerance test should be made to see if one is not dealing with a potential diabetic.—H. J. J.

On the nervous control of lactation. Selye, H., Am. J. Physiol. **107**: 535. 1934.

The main milk duct was cut at each of the nipples of seven lactating rats, three days after parturition. The skin incisions were made at some distance from the nipple and the main galactophore was approached through a channel made in the subcutaneous tissue, so that all possible injury to the nipples was avoided. After the operation the mothers were returned to their litters; as none of the nipples gave milk, the young nursed them all to the same extent. In order to keep the young alive they were placed with a normal mother on alternate days, the latter's own young being transferred on these days to the

experimental animal. Thus the operated animals were continuously exposed to the suckling stimulus, without emptying of the glands. In a control series the litters were removed from 10 mother rats on the third day of lactation, in order to see how soon the mammary gland would show signs of involution after the stimulus of suckling ceased. In two of these controls the galactophores were cut as in the experimental series—a further check which later proved to be unnecessary. The animals of both series were killed at various intervals, 3 to 14 days after the beginning of the experiment. Examination showed that in the entire first series, even those killed after 14 days, the mammary glands were turgid with a large amount of milk, while in the second series the glands contained no milk after 3 to 5 days, and in 6 to 8 days the alveoli of the gland began to disappear. The act of suckling maintains the mammary glands of the rat in the stage of active secretion over long periods, even though the galactophores are cut and the milk cannot be removed. Suckling at one set of nipples may maintain active secretion in other glands where suckling has been prevented by excision of the nipples. The physiological involution of the mammary gland after weaning is thus conditioned by the withdrawal of the nervous stimulation of suckling rather than by the accumulation of the products of secretion in the gland.—Author's Abst.

Excessive gonad-stimulating hormone and subnormal amounts of oestrin in the toxaemias of late pregnancy. Smith, G. V. S. and O. W. Smith, Am. J. Physiol. **107:** 128. 1934.

From 42 women in the last third of pregnancy 46 sera and 44 24-hour specimens of urine were analyzed for their content of the gonadotropic hormone (prolan) and oestrin. Of these women, 15 were normal, 22 had toxic symptoms without convulsions and 5 had eclampsia. One of the patients with toxic symptoms was definitely nephritic and showed normal levels of prolan and oestrin. In no case diagnosed as toxaemia or eclampsia were both oestrin and prolan within the limits of values found in the normal women at the same period of gestation. In a number of the toxaemic women, however, there was unquestionably a nephritic element. All but one of 26 toxaemic and eclamptic patients, or 96%, showed excessive amounts of prolan in the urine and serum, and 18 of them, or 96%, had subnormal levels of oestrin. The urines and sera of one toxaemic patient were analyzed monthly from the second month to term. During the sixth month an abnormal increase in prolan and decrease in oestrin appeared, which continued with fluctuations until delivery. This patient developed no toxic symptoms until the eighth month. It is concluded that a quantitative imbalance of these two hormones due to excessive amounts of prolan and, less consistently, to subnormal levels of oestrin is typical of the toxaemias of late pregnancy.—Authors' Summary.

Negative effect of prolonged administration of ovarian substances in hemophilia. Stetson, R. P., C. E. Forkner, W. B. Chew and M. L. Rich, J. A. M. A. **102:** 1122. 1934. Abst., A. M. A.

The authors observed the coagulation time of the blood of seven patients with hemophilia repeatedly during treatment with ovarian substance or estrogenic substance. Large amounts of one or another of eight different preparations were given over periods of from 28 to 81 days. In no instance was the coagulation time of the venous blood found to be depressed as the result of such therapy. Theelin (estrogenic substance) administered subcutaneously to two patients for from three to seven days neither stopped bleeding nor reduced the clotting time. Estrogenic substance was demonstrated consistently in the urines of the patients both under ovarian therapy and during control periods. No correlation could be established between the quantity of estrogenic substance excreted in the urine and the fluctuations of the blood coagulation time. No clinical improvement was noted which could be attributed to ovarian therapy. In four of the patients hemarthroses developed without obvious trauma or infection late in the course of treatment.

Ovogenesis and its relation to the hypophysis: the effects of pregnancy, hypophysectomy, thyroidectomy, and hormone administration on the ovary of the rat. Swezy, Olive, The Science Press, Pp. 86, Lancaster. 1933.

The ovaries of normal, pregnant, unilateral-ovariectomized pregnant, pseudopregnant, hypophysectomized, thyroidectomized, and hormone-treated rats were serially sectioned and examined for morphological details, the ova,

follicles, corpora, and cysts being counted in the entire ovary. These studies showed the following results. The hypophysis has no causal relation to ovogenesis: after hypophysectomy about twice as many ova are produced by the germinal epithelium as are found in the normal controls. In rats which had received hypophysial implants both ovogenesis and follicular growth had been depressed below the normal rate. An excess of luteinizing hormone produces larger lutein cells and corpora and prolongs their life span, at the same time inducing a rapid regression and complete disappearance of the older corpora, and induces the formation of lutein cells from the germinal epithelium and tunica albuginea. These effects are similar in pregnant and maturity-hormone-treated rats. More ova and small follicles are produced during pregnancy than in the normal, non-pregnant cycle. Mating, whether resulting in pregnancy or pseudopregnancy, produces an immediate rise in the rate of ovogenesis, the effects of the luteinizing hormone appearing from about the sixth day onward. Pheudopregnancy shows a slighter effect of maturity hormone than does pregnancy. Rats treated with growth hormone had ovaries like those of the hypophysectomized rat, normal ovaries, and ovaries extensively luteinized. There are no evidences of the presence of the maturity hormone in the ovary before about the eighteenth day. The corpora in the ovaries of rats receiving maturity hormone are formed mainly from the theca interna, the granulosa completely degenerating in many cases. When the granulosa contributes to the formation of lutein cells, these do not differ in size from those from the theca interna.—Author's Abst.

Studies on the physiology of reproduction in birds; body size changes in doves and pigeons incident to stages of the reproductive cycle. Riddle, O. and Pela Fay Braucher, Am. J. Physiol. **107**: 343. 1934.

The body weight of adult doves and pigeons undergoes a cyclic increase of about 8% in weight during the 15 or 18 days spent in the incubation of their eggs. Maximum weight is attained at the end of incubation. Nearly one-third of this increase occurs in the crop-glands under the specific stimulus of prolactin. The remainder of the increased weight seems to occur in the body in general except in the skeleton, it is now indistinguishable from true body growth, and is believed to have a hormonal basis which in time relations at least is associated with the release of prolactin.—Authors' Summary.

The physiologic maintenance of the male sex glands; the effect of androtin on hypophysectomized rats. Walsh, E. L., W. K. Cuyler and D. R. McCullagh, Am. J. Physiol. **107**: 508. 1934.

Hypophysectomized rats treated with male sex hormone from urine did not show the atrophy and histological changes in the testes, epididymis, prostate and seminal vesicles which are shown by untreated hypophysectomized animals. There was no protection by the male sex hormone against atrophy and histological changes in thyroid, suprarenal and thyroid glands in hypophysectomized rats.—M. O. L.

Gonadotropic hormones (p factors); preparation and properties of extracts of anterior pituitary, placenta, and pregnancy urine. Wiesner, B. P. and P. G. Marshall, Quart. J. Exper. Physiol. **21**: 147. 1931.

Consideration is given to methods of assay and detection of the various hormones in question. In the present experiments 26 to 28-day-old mice were used, and the authors believe that the use of sufficiently large numbers of animals obviates the possible error of spontaneous oestrous interfering with results. For the detection of oestrogenic activity oestral smears were made on the immature mice as soon as the vaginal orifice had opened. Mucinification was not considered as evidence of the presence of luteal activity but merely considered as evidence of some ovarian secretory activity. The authors stress the fact that the reaction of spayed adult females may not be sufficiently sensitive to rule out the presence of small quantities of oestrin which would be sufficient to produce some vaginal changes in the immature, intact animal. With alkaline extracts anterior pituitary luteinization was obtained in all dilutions and similar results were obtained with filtered extracts. Yet, deproteinized alkaline extracts proved to be oestrogenic and did not inhibit the cycle of mature animals. Extraction of cattle pituitaries with alcohol, acetone, and ether failed to produce any active extract. Despite the frequent

statements in the literature, the authors find that prolonged treatment of an active extract with alkali does not separate the luteinizing from the follicle-stimulating activity, but results in the diminution of potency in regard to both activities. In regard to boiling, it is now found that it merely reduces the potency of the extract, and does not separate the A and B factors as was formerly held by one of the authors (Wiesner). Attempts to extract an ovary-stimulating factor from the placenta by alcohol according to the method proposed by Collip (emmenin) yielded a product which would produce oestrus effects, but which was entirely inactive in regard to ovarian stimulation. The prolonged injection of purified fractions of the sulphosalycylic acid extract, formerly thought to be purely estrogenic, led to the inhibition of the cycle in mature animals and the production of corpora lutea in immature animals. To account for such results the authors prefer to hypothesize the existence of two hormones in the anterior lobe (luteinizing and follicle-stimulating), present in unequal amounts, and having a differential increment of effect with progressively larger doses, in which "rho ii" would have a greater increment of effect than "rho i," and, it is further suggested that the maximum effect of "rho ii" is greater than that to be obtained by "rho i." The chemical properties of the various extracts are described.—M. H. Friedman.

Thyroid and diuretic activity of anterior pituitary lobe extract (Tiroides y acción diurética del extracto de lóbulo anterior de la hipófisis). Biasotti, A., Rev. Soc. argent. de biol. **9:** 499. 1933.

Alkaline anterior pituitary extract (Evans and Simpson's) has a diuretic activity in normal dogs, independent of the diabetic condition it provokes. This response is not modified by castration nor section of the splanchnics. Thyroidectomy suppresses the polyuria. Extracts of spleen, muscle, kidney and liver do not produce diuresis, but ingestion of thyroid does. The diuretic activity of the anterior lobe extract is due to the state of hyperthyroidism it produces.—J. T. L.

Purine metabolism in hypophysectomized dogs (Metabolismo púrico del perro hipofisoprivo). Braier, B., Rev. Soc. argent. de biol. **9:** 365. 1933.

Three hypophysectomized and 3 normal dogs were studied. They were kept on a diet of 40 grams of lean meat per kg. body weight for 6-10 days. During a second period nitrogen was gradually diminished and replaced by starch, sugar and fat till the animals were in a complete protein fast for 4-5 days. In these three nutritive conditions hypophysectomized dogs eliminated 32-36% more allantoin than the normal controls. The sum of all purine bodies excreted (uric acid, purine bases, allantoin) was equal in both groups, whatever the diet. During protein fast the ratios allantoin nitrogen, total urinary nitrogen and total purine nitrogen: total urinary nitrogen in the hypophysectomized dogs were 2.5 and 2 times the value found in the normal controls. This is due to the lower total nitrogen and higher allantoin excretion in hypophysectomized animals. The puric oxidation coefficient is higher in hypophysectomized dogs.—J. T. L.

Perfusion of an "isolated" head alternately with blood from dog A and dog B. Hormonal influences coming from the head (Perfusion de la tete "Isolée" alternativement par un Chien A et un Chien B. Influences hormonales d'origine céphalique). Heymans, C., J. J. Bouckaert and L. Brouha, Compt. rend. Soc. de biol. **112:** 720. 1933.

A diagram is given showing the essentials of this method. Blood returning from the head has an inhibitory effect on kidney secretion and no effect on circulation.—J. C. D.

The relationship between the anterior pituitary and the reproductive glands (Beziehungen zwischen Hypophysenvorderlappen und Keimdrüsen). Hohlweg, W. and M. Dohrn, Wien. Arch f. inn. Med. **21:** 337. 1931.

The authors have confirmed previous experiments on the inter-relationship of the anterior pituitary body and the sex glands. As a result of castration, irrespective of age and sex of the animal, changes took place in the anterior pituitary in the appearance of the so-called "Castration Cells." They found that daily injections of 1 r.u. of Progynon for a period of three weeks immedi-

ately after castration prevented these changes in the anterior pituitary, and that daily injections of 1 r.u. of Progynon beginning three weeks after castration restored the anterior pituitary to normal. They also found that 1/30 r.u. daily injected in the female rat was the minimum dose required to prevent the appearance of "Castration Cells"; and in the male rat, that twelve times the amount was necessary. Furthermore, they found that 1/15 r.u. produced oestrus in the female rat. These investigators showed that the daily production of follicular hormone in the ovary of a 20-30 gm. infantile rat is between 1/30 to 1/15 r.u. In their implantation experiments, they found that ovaries implanted immediately after castration produced no effect. Whereas, ovaries implanted three weeks after castration produced definite effects. Thirty rats were castrated, 15 of these were immediately implanted with ½ infantile ovary in the kidney, and the remaining 15 were similarly implanted three weeks after castration. The first group did not show oestrus; 80% of the second group showed oestrus between the sixth and the eighth day. In the first group the implanted ovary assumed the function of the extirpated ovary maintaining hormonal balance; whereas, in the second group during the three-week interval, the anterior pituitary developed hyperactivity, activating the implanted ovary in the production of oestrus. Infantile rats implanted with the anterior pituitary body of castrated rats treated with Progynon for three weeks after castration did not produce oestrus. The macroscopic and microscopic examination of the genitals showed that they remained infantile. Rats implanted with the anterior pituitary body from a castrated, untreated animal did produce oestrus, and the genitals were markedly enlarged. Histologically the ovaries showed the presence of large graafian follicles.—B. Parvey.

Hyperglycemic action of posterior pituitary lobe extract (Acción hiperglucemiante del extracto de lóbulo posterior de la hipófisis). Houssay, B. A. and E. di Benedetto, Rev. Soc. argent. de biol. **9:** 300. 1933.

Dogs anaesthetized with chloralose received variable doses (1 to 2.5 and 5 to 10 mg. per kg.) of extract of standard powder of posterior pituitary gland. The injection was made intravenously, lasting 1 hour, 1 cc. of the solution being injected every minute. Blood sugar (Hagedorn and Jensen method) was determined up to 7 hours after the beginning of the experiment. An increase proportional to the dose injected was observed.—J. T. L.

Syphilis of the pituitary body; a case report with review of literature. Kennedy, F. S. and J. H. Fisher, Am. J. Syph. & Neurol. **18:** 12. 1934.

A gumma of the pituitary body occurring in a woman 58 years old, suffering from acquired syphilis, was found at autopsy. This is a rare manifestation of syphilis. No clinical evidence suggesting pituitary disease was noted in the patient. The importance of routinely investigating the pituitary body in all cases of syphilis at autopsy is stressed. Two figures, photomicrographs of the gumma, accompany the paper. The literature bearing on the subject is reviewed.—Authors' Summary.

Which cells of the human hypophysis form the pituitary sex hormone in absence of pregnancy (Welche Zellen der menschlichen Hypophyse bilden ausserhalb; der Schwangerschaft das Vorderlappengeschlechtshormon (VLGH)? Kraus, E. J., Klin. Wchnschr. **11:** 1020. 1932.

In two instances transplants of parts of eosinophile adenomas produced oestrus and correlated ovarian changes in immature mice. One basophile adenoma was similarly examined with similar results and a small piece of a chromophobe adenoma produced some follicular growth in the immature animal. The author advances these data to support the thesis that the eosinophiles and chromophobes partake in the formation of the gonadotropic hormone.—M. H. Friedman.

Anterior pituitary growth hormone and the composition of growth. Lee, M. O. and N. K. Schaffer, J. Nutrition **7:** 337. 1934.

The effect of the growth hormone of the anterior pituitary upon the gain in weight and upon the composition of the gain was determined in 12 pairs of rats under limited food intake. Twelve control animals showed characteristic and statistically significant changes in body composition with age—decreases

in the proportions of water, nitrogen, fat-free dry tissue and ash, and increases in the percentage of fat and in the heat value of the tissue. The 12 treated animals retained almost exactly their initial composition in all constituents and in the heat value of their tissue. Their excess gains over their control mates, on the same food consumption, were: live weight, 531 gm.; body length, 15 cm.; water, 447 gm.; nitrogen, 22.5 gm.; ash, 22.4 gm.; fat, —119 gm.; energy, —341 calories. Nitrogen and ash balances determined indirectly corroborated the direct findings from carcass analysis. The weekly excess nitrogen retention in the treated rats paralleled closely their weekly excess gain in weight. The energy cost was 1.7 times as great for the controls as for the treated animals, per gram of weight gained, and 2.4 times as great per gram of protein gained. The treated animals burned more fat and less protein than did the controls. Statistical analysis of the data indicates that the nitrogenous constituents were the ones most specifically influenced by the hormone.

—Authors' Abst.

Psychoses and tumors of the pituitary body (Psychosen bei Hypophysentumoren). Meumann, E., Arch. f. Psychiat. **96:** 609. 1932. Abst., Arch. Neurol. & Psychiat. **31:** 874.

The author presents two cases of acromegaly with pituitary tumors (in the first case proved by exploration and in the second by autopsy), in which mental disturbances were associated with the neurologic symptoms. These disturbances were of a chronic type and characterized by hallucinatory and delusional phenomena. Superficially they resembled the paranoid type of dementia praecox, but closer analysis showed definite differences. The development of these psychoses and their relationship to organic disturbances on the basis of the growth of the neoplasms are discussed.

Hypophyseal dysostosis (La dysostose hypophysaire; maladie de Schuller—syndrome de Christian; xanthomatose cranio-hypophysaire; reticulo-endotheliose cranio-hypophysaire). Moreau, J., Arch. franco-belges de chir. **32:** 697. 1931. Abst., Arch. Neurol. & Psychiat. **30:** 1154.

Moreau has gathered together 34 cases of the Schüller-Christian syndrome and reports in detail a case observed by him. The reports collected from the literature are presented in extremely detailed abstracts, practically complete case histories. The early reports of Schüller and of Christian, together with the synthetic work of Rowland, are discussed fully, and many roentgenograms are reproduced from the original publications. The disease is characterized by four peculiarities: osteoporosis of the skull, exophthalmos, polyuria and pituitary dwarfism. The pathogenesis is probably a disturbance of the lipoid metabolism, characterized by hypercholesteremia and deposits of a xanthomatous character in the bones, particularly of the skull, and in the infundibulum, and less frequently in the skin and the internal organs. The results of irradiation of the skull have been gratifying, and arrest of the condition or even recovery is reported. Spontaneous recovery occasionally occurs. Treatment by radiotherapy should be pursued diligently over a long period. In addition, a diet low in lipoids should be administered, and certain hypophyseal extracts are worthy trying.

Hypophyseal cachexia in a child (Hypophysäre Wachstumshemmung mit Kachexie beim Kinde). Prüsener, L., Ztschr. f. Konstitutionslehre, **17:** 215. 1932.

Organ weights, body proportions, ossification and histological findings are given of a 3½-year-old male child that had gained only 1600 grams since birth. The anterior lobe was greatly distended by a large colloid cyst surrounded partly by cuboidal and partly by flattened cells. While there were some eosinophilic and basophilic cells, the rim of anterior lobe tissue left was largely composed of chromophobes with evidence that the pressure had seriously interfered with hypophyseal function for a duration of probably 2½ years.

—A. T. R.

A case of Simmond's disease (cachexia hypophyseopriva) with recovery. Striker, C., Proc. Central Soc. Clin. Res., J. A. M. A. **101:** 1994. 1933.

A white girl, aged 17 years, whose weight on admission was 48 pounds (22 kg.), had a basal metabolic rate of —48, and a slight secondary anemia. A roentgenogram of the sella showed suggestive cystic degeneration. Roent-

genograms of other bones showed marked calcium deficiency. The patient had been on a regimen of thyroid and ovarian extract prior to the examination, without any improvement. She had very marked abdominal cramps with constipation and had a well-marked cathartic addiction, taking as many as fifteen "Feen-a-Mint" tablets and three enemas daily without blood diarrhea. She was placed on 1 cc. of soluble extract from the anterior lobe of the pituitary hypodermically, daily, and continued on this for four months, with very striking improvement. Examination shows that the patient now weights 135 pounds (61 kg.), has been completely rehabilitated and is apparently cured of the intestinal condition.

Anterior hypophyseal hormones; the secretion of the follicle-stimulating hormone in the menstrual cycle; the influence of ovarian transplantation and the sex-hormone on the secreton of follicle-stimulating hormone after castration (Über die Hormone des Hypophysenvorderlappen; die Ausscheidung des Follikelreifungshormons [HVH-A] im mensuellen Cyclus); der Einfluss der Ovarialtransplantation und der Sexualhormone aif die Ausscheidung des Follikelreifungshormons [HVH-A] nach Kastration). Zondek, B., Klin. Wchnschr. **10:** 2121. 1931.

The author has analyzed the content of the follicle-stimulating hormone in the urine of women at various stages of the normal menstrual cycle. He found 8, 25, 29 and 25 rat units per day in the post-menstrual, inter-menstrual, pre-menstrual and menstrual stages, respectively, and 743 rat units per menstrual cycle. In the animals there was no production of Prolan A after the follicle had ruptured. The author points out the importance of realizing that our results of hormone-experiments on animals may not be directly applicable to humans. Daily analysis of the urine for hormones were made on a 31-year-old female before and after the removal of the uterus and right adnexa for an ovarian tumor. The left adnexa had been removed 10 years previously. Before operation—normal folliculin content, Prolan A not discernible; 18 days afterwards Prolan A was found for the first time (110 mouse units per liter of urine) and this quantity continued for 9 months without diminution. Homeoplastic ovarian-transplant into the space of Retzius was without effect on the urine hormones. Administration of folliculin sub-cutaneously and corpus-luteum intra-muscularily was without effect. In one instance, when folliculin to the extent of 2000 mouse units was given intravenously in 1 day, there was an increase of Prolan A from 110 to 200 mouse units per liter of urine.—R. A. Phillips.

The liver in muscle glycogen revovery after fatigue (Papel del higado en la reconstitucion del glycogeno muscular despues de la fatiga). Dambrosi, R. G., Rev. Soc. argent. de biol. **9:** 413. 1933.

Hepatectomized dogs did not restore muscle glycogen after fatigue when the blood sugar was low, but a slight recovery was observed when the blood sugar level was still high. Intravenous injection of 1 gram glucose per kg. immediately after fatigue in these animals allowed a normal glycogen restorage. The importance of the liver resides in its rôle as a factor maintaining the normal blood sugar level.—J. T. L.

Pilocarpine and insulin secretion. Appelrot, S., Am. J. Physiol. **107:** 526. 1934.

Large doses of pilocarpine produce in normal dogs a slight rise, and in pancreatectomized dogs a very pronounced rise in blood sugar. It is concluded that pilocarpine stimulates the islet tissue of the pancreas, but that this effect is overshadowed by other antagonistic mechanisms such as stimulation of the adrenal and possibly the thyroid glands.—M. O. L.

The resistance of surviving spinal animals to hypoglycemia induced by insulin. Brooks, C. M., Am. J. Physiol. **107:** 577. 1934.

The 27 cats, with cords transected at the level of the sixth cervical vertebra, were kept for over a week before being used in these experiments. That was a sufficient time for the return of arterial pressure to normal. Vigorous cross-extension and flexion reflexes indicated rather complete recovery from spinal shock. Varying doses of insulin were injected subcutaneously. These animals had a diminished resistance to insulin for doses of from 2-3 units per

kgm. were always fatal. The hypoglycemia activated the sympathico-adrenal system, however, to such an extent that 0.5 units per kgm. was never fatal and usually a cat recovered from 1 unit per kgm. The spinal animals and the normal animals in which the adrenals had been inactivated did not recover spontaneously from 0.5 unit of insulin per kgm. Using the chronically denervated heart as an indicator it was possible to detect a discharge of adrenine when the blood sugar reached a critical hypoglycemic level in the spinal preparations. Convulsions were eliminated by cervical transection of the cord though other hypoglycemic symptoms occurred. These observations indicate that a hypoglycemia induced by insulin, which does produce strong sympathico-adrenal activity in the normal cat, can likewise induce activity of this system in a spinal cat in which all nervous connections between higher centers and the spinal thoroco-lumbar sympathetic outflow have been cut. The results afford further evidence for the existence of a spinal mechanism capable of exerting influence upon the peripheral sympathico-adrenal system.
—Author's Abst.

Pancreas and muscle glycogen (Pancreas y formación de glucógeno muscular). Foglia, V. G. and R. Fernández, Rev. Soc. argent. de biol. **9:** 506. 1933.

Experiments were made on chloralosed dogs with sciatic nerves cut. Blood sugar by the Hagedorn-Jensen method and glycogen of tibialis anticus, common extensor or gastrocnemius by Pflüger's method were determined. In normal resting muscle the glycogen content varied between 300 and 600 mg. per cent. Intravenous injection of glucose (1-2 gm./kg.) produced an increase in muscle glycogen proportional to the dose given, most marked 1½ hours after injection; there was no definite relation between the rate of glycogen formation and return of blood sugar to normal. Insulin (4 units per kg.) increased this storage of glycogen. In pancreatectomized dogs (immediately, 5 hours and 24 hours after operation) no storage of glycogen in muscle occurred after glucose injection unless insulin was given. A vascular graft of the pancreas made into the carotid and jugular vein reëstablished the normal capacity of muscle for glycogen storage on glucose injection. Pancreatectomized dogs had approximately normal resting muscle glycogen values up to 24 hours after operation. In the absence of insulin muscle glycogen fixation is decreased or retarded. The glycogen fixing function of insulin secretion can be performed by the denervated pancreas.—J. T. L.

The vagi in muscle glycogen formation (Papel de los vagos en la formación del glucógeno muscular). Foglia, V. G. and R. Fernández, Rev. Soc. argent. de biol. **9:** 517. 1933.

Experiments were performed on dogs under chloralose. Double vagotomy in the neck did not modify the capacity of muscle to store glycogen after intravenous injection of 2 gm./kgm. of glucose, immediately and 5 hours after the operation. After an interval of 24 hours sometimes there was less glycogen fixation by muscle, but the animals were in a precarious condition. When artificial respiration was given and vagotomy was performed at the level of the cardia glycogen fixation was more marked.—J. T. L.

Hyperinsulinism and epilepsy; presentation of patients and case reports. Harris, S., South. M. J. **26:** 1026. 1933.

Six cases are presented, four showing reduced blood sugar with epilepsy, one with narcolepsy and low blood sugar, and one with hypopituitarism, hyperinsulinism and epilepsy. Maintaining the blood sugar as near normal as possible was of marked benefit.—J. C. D.

Differential diagnosis of diabetes by constitutional signs (Beiträge zur differentialdiagnose des Diabetes mellitus nach Konstitutionellen Merkmalen). Horsters, H., Ztschr. f. d. ges. exper. Med. **80:** 86. 1933.

The author stressed the differentiation of the fat from the emaciated diabetic as two distinct constitutional types, and attempted to make a differential diagnosis of the two types from the following laboratory studies: Blood sugar response from 200 grams of white bread and 1500 cc. tea over a period of four hours; 200 grams of meat and 1500 cc. tea; 200 grams sweet cream, a small amount of bread and 1500 cc. tea. The investigation was carried out on more than 400 diabetics over a period of 4 years. The patients were grouped

as follows: Young, emaciated diabetics with low blood pressure; fat diabetics with high blood pressure; diabetics with elevated blood pressure and familial obesity; emaciated forms of formerly fat diabetics with high blood pressure; one case of a youthful diabetic with low blood sugar. In normal patients on the above routine, there is fluctuation of blood sugar which is similar. The peroral glucose, in repeated experiments, varies somewhat in the type of a curve presented, yet there is definite resemblance of the curves. In patients with disturbed metabolism (diabetics) the rise of blood sugar after glucose is quite high. From the curves alone one cannot identify the patient. The massive dose leads to high blood sugar curves but these curves retain their identity over short periods, from which the author thinks he could differentiate the various types of diabetes. In the four-day period of the various tests, the curves obtained give a picture of the protein and fat metabolism disturbances which is quite distinct from that of the normals. As a differential diagnosis between the emaciated and the fat diabetics the rise of blood sugar after a carbohydrate load is of great significance. The thin diabetic with low blood pressure after a carbohydrate load shows a slow rise of blood sugar, rising up to 400 mg. per cent within the first hour. In most cases there is no marked fall after the first hour but the level is retained over a period of hours, falling first toward the end of the fourth hour, but does not reach the original value. In the obese diabetics with high blood pressure, there is a contrasting slower rise of blood sugar, with its peak of 300 mg. rarely above this. Right after the first hour the blood sugar begins to fall, reaching its original level after four hours. Diabetics with familial obesity react likewise. The emaciated but formerly fat diabetics react similarly with the exception that the drop at or after the fourth hour is lower than the original value. The curves after a protein load in all high blood pressure cases show a steady fall of blood sugar. The form with low pressure of the youthful type show a rise of blood sugar after a protein load, whereas the fat load shows a rise of blood sugar only in the emaciated diabetics. The practical application for the differential diagnosis of the type of diabetes mellitus is the fasting blood sugar curve. All diabetics in this series with high blood pressure show a fall of blood sugar. Youthful diabetics with a low blood pressure show a rising curve. Studies in the quantity of urine and variations of specific gravity are given and these the author utilizes for the evaluation of renal function.—H. J. J.

Studies in diabetes mellitus; its incidence and the factors underlying its variations. Joslin, E. P., L. I. Dublin and H. H. Marks, Am. J. M. Sc. **187:** 433. 1934.

Consideration of the available material on incidence of diabetes and the factors underlying variations in its frequency reveals these significant facts. The number of known diabetics in this country is probably between 300,000 and 400,000. This estimate takes into account current diabetes mortality, the duration of life of diabetics, and the incidence of diabetes recorded in the various sickness surveys. A large increase in the incidence of diabetes is indicated by comparison of earlier surveys with recent ones, and also by hospital statistics. The incidence of diabetes abroad seems to be lower than in this country. Here the case incidence is about 2½ to 3 per M, compared to 1½ to 2½ in the various countries of Western Europe. The number of diabetics in Germany is estimated as 100,000-150,000 and in England as 50,000-75,000. Estimates for the other large countries cannot be made, because data necessary for computing them are not available. Mortality statistics show that 2½ million persons (2.08% of the present population) may be expected ultimately to succumb to the disease. The chance of eventually dying from diabetes are nearly twice as high for females as for males, and are higher for white persons than colored persons. Diabetes is more prevalent and seems to be increasing faster in urban areas than in rural ones. In this country, the northeastern states have a much higher diabetes death rate than those in the south. Average rates prevail in the middle west and on the Pacific Coast. This variation is greater in the rural than in the urban population. In Europe diabetes rates are relatively high in the north and west and low in the south. The Teutonic peoples suffer more frequently from diabetes than the Latins. The data on Czechoslovakia, if typical, indicate a low diabetes rate among the Slavic peoples. National and racial tendencies in this country conform largely to those abroad, except among the Irish, who, in Ireland, have a very low diabetes rate, but a high rate in this country. Recent studies of mortality in New York bring out clearly the excessive frequency of diabetes in Jews and confirm

earlier clinical observations. This finding does not seem to apply to young Jews. Among the older ones, the incidence is probably 1½ to twice the average. Diabetes is more frequent in the higher than in the lower social classes. The employing and professional groups tend to have high diabetes rates. This is also true of those who sell or serve food and drink. Diabetes is least frequently found in persons engaging in occupations requiring hard manual labor. Long sustained wars result in decreases in the diabetes rate, largely as a consequence of the reduction of food supplies available to civilians. For a variety of reasons the effect of economic crises is not altogether clear-cut, but in any case, it is less direct and less influential than the effect of war conditions. Sugar consumption in itself is not an important factor in the diabetes rate, nor in the increasing incidence of the disease. In the judgment of the authors the several external factors influencing the incidence of diabetes, which are the subject of the present article, depend upon a very few basic forces.

—Authors' Summary.

Effect of pancreatectomy on glutathione content of blood. Kunde, M. M., T. J. Becker, F. S. Saruk and R. D. Kearney, Proc. Soc. Exper. Biol. & Med. **31**: 535. 1934.

Complete pancreatectomy in the dog causes an increase of over 100% in the non-glucose reducing substance of venous blood. Section of the splanchnics in the dog causes a decrease of approximately 50% in the blood glutathione, but no effect on the experimental diabetes following pancreatectomy. Acetone given by mouth in dosages of 6 cc. per kilo of body weight causes this glutathione-like substance to increase approximately 200% above normal and does not return to normal for approximately 3 days.—M. O. L.

The spinal parasympathetic; influence of the spinal parasympathetic upon the internal secretion of the pancreas. Kuré, K., T. Tanishima and S. Okinaka, Quart. J. Exper. Physiol. **22**: 329. 1933.

In a series of 32 dogs, 17 experimental and 15 controls, the authors opened the abdomen under local anaesthesia, cut the splanchnic nerves and vagi, ligated the suprarenal veins and painted the coeliac ganglion with 2% nicotine. The suprarenal vein ligation prevents adrenaline from entering the circulation. By painting the coeliac ganglion with nicotine, the authors believe they enable only the action of parasympathetic fibers to become apparent. Samples of blood and sections of liver were taken in both the experimental and control groups. Blood sugar was determined directly and the amount of liver glycogen was gauged by histological examination of stained sections of liver. In the experimental group the splanchnics were stimulated for forty minutes. Blood samples were taken during and after the period of stimulation until the death of the animal. Liver samples were taken at the end of the experiment. The authors found what they believe to be a marked drop in the blood sugar of the experimental group, although the controls show a fairly progressive diminution in blood sugar. Histologically they found an increase in liver glycogen of the experimental group which they attribute to an increased secretion of insulin by the pancreas consequent to stimulation of spinal parasympathetic nerves.—I. A. Mirsky.